(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
3 October 2002 (03.10.2002)

PCT

(10) International Publication Number
WO 02/076503 A1

(51) International Patent Classification[7]: A61K 39/40, 39/42, 39/395, C07K 16/00, C12N 5/00, 5/02, C12P 21/08

(21) International Application Number: PCT/US01/19584

(22) International Filing Date: 19 June 2001 (19.06.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/212,577	20 June 2000 (20.06.2000)	US
60/269,788	16 February 2001 (16.02.2001)	US
60/287,171	27 April 2001 (27.04.2001)	US

(63) Related by continuation (CON) or continuation-in-part (CIP) to earlier applications:

US	60/212,577 (CON)
Filed on	20 June 2000 (20.06.2000)
US	60/269,788 (CON)
Filed on	16 February 2001 (16.02.2001)
US	60/287,171 (CON)
Filed on	27 April 2001 (27.04.2001)

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
with international search report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/076503 A1

(54) Title: TREATMENT OF CENTRAL NERVOUS SYSTEM DISEASES BY ANTIBODIES AGAINST GLATIRAMER ACETATE

(57) Abstract: The present invention provides humanized polyclonal and humanized monoclonal antibodies directed against an epitope on glatiramer acetate, also known as Copolymer 1, Copolymer-1, Cop-1 or Cop. Additionally, the subject invention concerns a pharmaceutical composition comprising an antibody directed against an epitope on glatiramer acetate for the treatment of a disease associated with demyelination of central nervous system axons. Also encompassed by the subject invention is a method of treating a subject suffering from a disease associated with demyelination of central nervous system axons. The subject invention further contains methods of stimulating remyelination of central nervous system axons. In addition, the subject invention provides a method of stimulating proliferation of lymphocytes.

TREATMENT OF CENTRAL NERVOUS SYSTEM DISEASES BY ANTIBODIES AGAINST GLATIRAMER ACETATE

Throughout this application, various references are referenced by arabic numbers within parenthesis. Full citations for these references may be found at the end of the specification, immediately preceding the claims. These references, in their entireties, are hereby incorporated by reference to more fully describe the state of the art to which this invention pertains.

Field of the Invention

The present invention is directed to the treatment of central nervous system (CNS) diseases by antibodies.

Background of the Invention

The nervous system of vertebrates is divided into the central nervous system, comprised of the brain and spinal cord, and the peripheral nervous system, consisting of the outlying nerves (11). The axons of most nerve cells are covered with a myelin sheath, a stack of specialized plasma membranes. Glial cells that wrap around the axons produce the myelin sheath. In the CNS, these cells are called oligodendrocytes. The myelin membranes of the CNS contain myelin basic protein (MBP) and a proteolipid (PLP) that is not found elsewhere in vertebrates. Each region of myelin formed by an individual glial cell is separated from the next region by an unmyelinated area called the node of Ranvier; only at nodes is the axonal membrane in direct contact with the extracellular fluid.

The myelin sheath, which can be 10-12 myelin wraps thick, acts as an electric insulator of the axon by preventing the transfer of ions between the axonal cytoplasm and the extracellular fluids (11). Thus all electric activity in axons is confined to the nodes of Ranvier, the sites where

ions can flow across the axonal membrane. Node regions contain a high density of voltage-dependent Na+ channels, about 10,000 per μm^2, whereas the regions of axonal membrane between the nodes have few if any channels.

The excess cytosolic positive ions generated at a node during the membrane depolarization associated with an action potential diffuse through the axonal cytoplasm to the next node with very little loss or attenuation because ions are capable of moving across the axonal membrane only at the myelin-free nodes (11). Thus a depolarization at one node spreads rapidly to the next node, and the action potential "jumps" from node to node. For this reason, the conduction velocity of myelinated nerves is much greater than that of unmyelinated nerves of the same diameter. For example, a 12-μm-diameter myelinated vertebrate axon and a 600-μm-diameter unmyelinated squid axon both conduct impulses at 12m/s.

One of the more common neurologic diseases in human adults is multiple sclerosis. This condition is a chronic, frequently progressive, inflammatory CNS disease characterized pathologically by primary demyelination. The etiology and pathogenesis of multiple sclerosis are unknown. Researchers have hypothesized that multiple sclerosis is an autoimmune disease (9, 13, 24) or that a virus, bacteria or other agent, precipitates an inflammatory response in the CNS, which leads to either direct or indirect ("bystander") myelin destruction, potentially with an induced autoimmune component (16, 19). Thus, a rebuilding of the myelin sheath, or remyelination, can treat multiple sclerosis.

Spontaneous remyelination of axons within lesions by oligodendrocytes has been shown to occur to a small degree in SJL/J mice and multiple sclerosis patients (1). Several types of antibodies have been found to promote remyelination (1). Some of these antibodies are polyclonal, derived by

immunization with spinal cord homogenate or myelin basic protein (39). One remyelination-promoting antibody is monoclonal (SCH 94.03) (1). The isotype of these antibodies is IgM, and they share the characteristic of binding to the surface of oligodendrocytes (1). Also, they are polyreactive, binding to a variety of cytoskeletal proteins or proteins with repeating structures (1).

Of clinical importance is the question whether morphologic regeneration of thin myelin sheaths contributes to functional recovery (1). Computer simulations indicate that new myelin formation even by inappropriately thin sheaths improves impulse conduction (1). Since the axon membrane of normally myelinated fibers is highly differentiated, it is necessary for sodium channels to be present at high density at the node of Ranvier to propagate saltatory conduction. Experimental evidence suggests that newly formed nodes do develop the required high sodium channel density as demonstrated by saxitoxin binding. Data suggest that remyelination even by inappropriately thin myelin improves conduction in a previously demyelinated axon. Therefore, any strategy to promote this morphologic phenomenon has the potential of producing functional recovery. Studies examining biopsy tissues from patients with severe acute exacerbations demonstrate that remyelination is a significant component of the acute multiple sclerosis lesion (33). Therefore, remissions are probably associated with significant CNS remyelination (1).

One commonly utilized experimental model of multiple sclerosis is induced by Theiler's murine encephalomyelitis virus (TMEV) (10, 29). Previous experiments in Strain Jackson Laboratories (SJL) mice infected with TMEV showed that 4 to 5% of the demyelinated area exhibited significant spontaneous remyelination (30). In protocols using antibody therapy and monoclonal antibody therapy (23, 32, 39), this

number increased up to 30-35%. For instance, using the TMEV model, it was demonstrated that the passive transfer of CNS specific antiserum (31) and purified antibodies (30, 27, 39) directed against myelin components promoted CNS remyelination. This contrasts with the conventional view that the humoral immune response plays a pathogenic role in CNS demyelination (28). Researchers also generated a monoclonal antibody that reacted against a surface component of oligodendrocytes and promoted remyelination (21 - 23). It has also been shown that antibodies reactive with myelin basic protein (MBP) promoted CNS remyelination (32). In these experiments, infected SJL mice were treated with the whole anti-serum or affinity purified mouse antibodies directed against rabbit or rat myelin basic proteins. There was extensive evidence for new myelin synthesis as measured by quantitative morphometry. Electron microscopy revealed numerous oligodendrocytes and remyelinated CNS axons with a relative lack of inflammatory cells. Viral antigen persisted in these animals despite enhanced CNS remyelination. These findings indicated for the first time that antibodies reactive against a myelin autoantigen and in particular, MBP, have the potential for myelin repair.

U.S. Patent 5,591,629 describes the promotion of CNS remyelination in the TMEV model through SCH 94.03 monoclonal antibodies directed against spinal cord homogenate (SCH)(1). SCH encompasses myelin antigens, such as MBP (34) and proteolipid protein (PLP) (8, 35). Although SCH contains MBP, this antibody does not react with MBP. The SCH 94.03 antibody is an IgM which recognizes cytoplasmic determinants on glial cells. It also recognizes surface determinants on glial cells, including oligodendrocytes. Experiments demonstrated that the antibody does not react with TMEV. In addition, the antibody was shown to promote the proliferation of glial cells in mixed rat brain culture in a dose-dependent manner. SCH 94.03 is a natural autoantibody.

Another treatment that has been shown to be effective in reducing exacerbations of multiple sclerosis is the administration of glatiramer acetate (2 - 6, 16). COPAXONE® is the brand name for glatiramer acetate (also known as Copolymer-1 (43), Copolymer 1, Cop-1 or Cop), an FDA-approved drug for the treatment of multiple sclerosis. Glatiramer acetate, the active ingredient of COPAXONE®, consists of the acetate salts of synthetic polypeptides, containing four naturally occurring amino acids: L-glutamic acid, L-alanine, L-tyrosine, and L-lysine with an average molar fraction of 0.141, 0.427, 0.095, and 0.338, respectively (43). The average molecular weight of glatiramer acetate is 4,700 - 11,000 daltons (43). Chemically, glatiramer acetate is designated L-glutamic acid polymer with L-alanine, L-lysine and L-tyrosine, acetate (salt) (43). Its structural formula is:

$$(Glu, Ala, Lys, Tyr)_x \cdot XCH_3COOH$$
$$(C_5H_9NO_4 \cdot C_3H_7NO_2 \cdot C_6H_{14}N_2O_2 \cdot C_9H_{11}NO_3)_x \cdot XC_2H_4O_2$$
CAS - 147245-92-9

(43).

Antisera against glatiramer acetate have been employed to investigate the mechanism by which L-glatiramer acetate is effective against Experimental Allergic Encephalomyelitis (EAE) (41, 42). For this purpose, Webb et al. measured the cross-reactivity of L-glatiramer acetate anti-sera with D-glatiramer acetate and Copolymer 4 (L-glatiramer acetate modified by the replacement of tyrosine with tryptophan) (42). Webb et al. carried out a similar experiment to determine the reactivity of L-glatiramer acetate anti-sera with L-glatiramer acetate, and the cross-reactivity of L-glatiramer acetate anti-sera with AGT (alanine, glutamic acid and tyrosine), BE (Basic Encephalitogen), AAspLT (alanine, aspartic acid, lysine and tyrosine) and AGL (alanine, glutamic acid and lysine) (41).

Monoclonal antibodies against glatiramer acetate and against MBP have also been utilized to probe the mechanism of glatiramer acetate in treatment of EAE (36). The cross-reactivity of monoclonal antibodies against glatiramer acetate with MBP was analyzed by Teitelbaum et al (36). They also determined the cross-reactivity of monoclonal antibodies against MBP with glatiramer acetate (36). Another focus of their experiments was the cross-reactivity of glatiramer acetate anti-sera with MBP and of MBP-antisera with glatiramer acetate (36). The cross-reactivity of anti-MBP anti-sera with glatiramer acetate was additionally investigated by Lisak et al (18).

Summary of the Invention

The subject invention concerns a humanized antibody directed against an epitope on glatiramer acetate, also known as Copolymer 1, Copolymer-1, Cop-1 or Cop.

The subject invention further encompasses a F_{ab} fragment that binds to an epitope on glatiramer acetate.

In addition, the subject invention relates to a pharmaceutical composition comprising an antibody directed against an epitope on glatiramer acetate in an amount effective to treat a central nervous system disease and a pharmaceutically acceptable carrier.

The subject invention also provides a method of stimulating remyelination of central nervous system axons comprising contacting the axons with an antibody directed against an

epitope on glatiramer acetate in an amount effective to stimulate remyelination of central nervous system axons.

The subject invention additionally includes a method of treating a subject suffering from a disease associated with demyelination of central nervous system axons comprising administering to the subject an effective amount of an antibody directed against an epitope on glatiramer acetate in an amount effective to treat the disease associated with demyelination of central nervous system axons.

The subject invention further relates to a method of stimulating remyelination of central nervous system axons comprising contacting the axons with glatiramer acetate in an amount effective to stimulate remyelination of central nervous system axons.

The subject invention also concerns a method of treating a subject suffering from a disease associated with demyelination of central nervous system axons comprising administering to the subject glatiramer acetate in an amount effective to treat the disease associated with demyelination of central nervous system axons, wherein the disease associated with demyelination of central nervous system axons is selected from the group consisting of: acute disseminated encephalomyelitis, transverse myelitis, demyelinating genetic diseases, spinal cord injury, virus-induced demyelination, Progressive Multifocal Leucoencephalopathy, Human Lymphotrophic T-cell Virus I (HTLVI)-associated myelopathy, and nutritional metabolic disorders.

Finally, the subject invention encompasses a method of stimulating proliferation of lymphocytes comprising contacting the lymphocytes with an antibody directed against an epitope on glatiramer acetate in an amount effective to stimulate lymphocyte proliferation.

Description of the Drawings

Figure 1 demonstrates that glatiramer acetate does not alter the extent of spinal cord demyelinating lesions during early disease. Each dot represents one mouse.

Figure 2 shows that glatiramer acetate at high doses increases the extent of spinal cord demyelinating lesions during late disease. Each dot represents one mouse.

Figure 3 reveals that glatiramer acetate does not alter the extent of remyelination during late disease. Each dot represents one mouse.

Figure 4 shows that glatiramer acetate does not alter the extent of brain pathology during late disease. Each dot represents one mouse.

Figure 5 demonstrates that antibodies against epitopes on glatiramer acetate do not alter the extent of spinal cord demyelinating lesions during late disease. Each dot represents one mouse.

Figure 6 reveals that antibodies against epitopes on glatiramer acetate promote remyelination during late disease. Each dot represents one mouse.

Figure 7 depicts the glatiramer acetate IgG develcped by non-infected mice after immunization.

Figure 8 shows that chronically diseased mice develop antibodies against epitopes on glatiramer acetate in response to glatiramer acetate treatment and that levels of these

antibodies are increased by co-administration with incomplete Freund's adjuvant (IFA).

Figure 9 demonstrates that the levels of antibodies against epitopes on glatiramer acetate increase as a function of time post-immunization and as a function of dose in chronically diseased mice.

Figure 10 reveals that glatiramer acetate IgG is detectable in serum 10 days after passive transfer to chronically diseased, non-immunized mice. Individual symbols represent means (± SEM) from 4-6 mice, except the untreated group (2 mice).

Figure 11 shows that affinity-purified antibodies against epitopes on glatiramer acetate (IgG and IgM) have high reactivity to Cop-1 by ELISA, whereas Normal antibodies have no reactivity to glatiramer acetate.

Figure 12 depicts the low polyreactivity of antibodies against epitopes on glatiramer acetate and Normal antibodies. In the figure, Hu=human; Ms=mouse; Rb=rabbit; Bov=bovine; BSA=bovine serum albumin.

Figure 13 shows that antibodies against epitopes on glatiramer acetate and Normal antibodies have a wide spectrum of antibody isotypes.

Figures 14-16 reveal that glatiramer acetate anitbodies stimulate proliferation of lymph node-derived lymphocytes from mice immunized with myelin peptides but not from non-immunized mice. The quantities shown are per 200 μl medium per well.

Figures 17-18 demonstrate that antibodies against epitopes on glatiramer acetate bind to microglia and macrophages, but not to oligodendrocytes. Figures 18-A - 18-D show the staining of antibodies against glatiramer acetate, while Figures 18E -

18-F reflect the staining of O4-positive oligodendrocytes.

Figures 19 and 20 display demyelinating lesions from chronically diseased, phosphate buffered saline (PBS)-treated mice.

Figures 21 and 22 portray demyelinating lesions from chronically diseased, glatiramer acetate-treated mice (0.1 mg/injection). Lesions show attempts at remyelination.

Figure 23 and 24 illustrate demyelinating lesions from chronically diseased, glatiramer acetate antibody-treated mice. Lesions show extensive remyelination (outlined), characterized by thin myelin sheaths around axons.

Figure 25 explains the process of purifying antibodies against epitopes on glatiramer acetate.

Figure 26 outlines the steps by which Normal antibodies are purified.

Detailed Description of the Invention

The phrase, "Early disease", is defined as the period up to 45 days post-infection and encompasses the encephalitic stage of disease as well as the beginning of the demyelinating phase of disease (begins at approximately Day 21 post-infection).

The phrase, "Late disease", is defined as the period beyond four months of infection.

The phrase, "Low-dose glatiramer acetate", is defined as single or multiple injections of glatiramer acetate at 0.02-0.1 mg/injection. Given the average weight of adult SJL/J mice of 20 g, a 0.1 mg injection is equivalent to 5 mg/kg.

The phrase, "High-dose glatiramer acetate", is defined as

single or multiple injections of glatiramer acetate at 5 mg/injection. Given the average weight of adult SJL/J mice of 20 g, a 5 mg injection is equivalent to 250 mg/kg.

The subject invention provides a humanized antibody directed against an epitope on glatiramer acetate.

In one embodiment, this humanized antibody is not cross-reactive with MBP.

In another embodiment, this humanized antibody consists essentially of IgG1.

In a further embodiment, this humanized antibody does not react with mature oligodendrocytes.

In another embodiment, this humanized antibody cross-reacts with SCH.

In one embodiment, this humanized antibody primarily reacts with cells exhibiting a macrophage or microglial phenotype.

In yet another embodiment, this humanized antibody is a monoclonal antibody.

In a further embodiment, this humanized antibody is a polyclonal antibody.

The subject invention further relates to a F_{ab} fragment that binds to an epitope on glatiramer acetate.

In addition, the subject invention involves a pharmaceutical composition comprising an antibody directed against an epitope on glatiramer acetate in an amount effective to treat a demyelinating central nervous system disease and a

pharmaceutically acceptable carrier.

In the pharmaceutical composition, this antibody may be a humanized antibody.

In the pharmaceutical composition, this antibody may be not cross-reactive with MBP.

In the pharmaceutical composition, this antibody may consist essentially of IgG1.

In the pharmaceutical composition, this antibody may not react with mature oligodendrocytes.

In the pharmaceutical composition, this antibody may cross-react with SCH.

In the pharmaceutical composition, this antibody may primarily react with cells exhibiting a macrophage or microglial phenotype.

In the pharmaceutical composition, this antibody may be a monoclonal antibody.

In the pharmaceutical composition, this antibody may be a polyclonal antibody.

The subject invention also provides a method of stimulating remyelination of central nervous system axons comprising contacting the axons with an antibody directed against an epitope on glatiramer acetate in an amount effective to stimulate remyelination of central nervous system axons.

In this method, the antibody may be a humanized antibody.

In this method, the antibody may be not cross-reactive with

MBP.

In this method, the antibody may consist essentially of IgG1.

In this method, the antibody may not react with mature oligodendrocytes.

In this method, the antibody may cross-react with SCH.

In this method, the antibody may primarily react with cells exhibiting a macrophage or microglial phenotype.

In this method, the antibody may be a monoclonal antibody.

In this method, the antibody may be a polyclonal antibody.

Additionally, the subject invention concerns a method of treating a subject suffering from a disease associated with demyelination of central nervous system axons comprising administering to the subject an effective amount of an antibody directed against an epitope on glatiramer acetate in an amount effective to treat the disease associated with demyelination of central nervous system axons.

In this method, the antibody may be a humanized antibody directed against an epitope on glatiramer acetate.

In this method, the antibody may not be cross-reactive with MBP.

In this method, the antibody may consist essentially of IgG1.

In this method, the antibody may not react with mature oligodendrocytes.

In this method, the antibody may cross-reacts with SCH.

In this method, the antibody may primarily react with cells exhibiting a macrophage or microglial phenotype.

In this method, the antibody may be a monoclonal antibody.

In this method, the antibody may be a polyclonal antibody.

In this method, the disease associated with demyelination of central nervous system axons is selected from the group consisting of: multiple sclerosis, acute disseminated encephalomyelitis, transverse myelitis, demyelinating genetic diseases, spinal cord injury, virus-induced demyelination, Progressive Multifocal Leucoencephalopathy, Human Lymphotrophic T-cell Virus I (HTLVI)-associated myelopathy, and nutritional metabolic disorders.

In one embodiment, the disease associated with demyelination of central nervous system axons is multiple sclerosis.

In another embodiment, the disease associated with demyelination of central nervous system axons is acute disseminated encephalomyelitis.

In an additional embodiment, the disease associated with demyelination of central nervous system axons is transverse myelitis.

In a further embodiment, the disease associated with demyelination of central nervous system axons is a demyelinating genetic disease.

In yet another embodiment, the disease associated with demyelination of central nervous system axons is a spinal cord injury.

In a further embodiment, the disease associated with demyelination of central nervous system axons is virus-induced demyelination.

In another embodiment, the disease associated with demyelination of central nervous system axons is Progressive Multifocal Leucoencephalopathy.

In an additional embodiment, the disease associated with demyelination of central nervous system axons is HTLVI-associated myelopathy.

In another embodiment, the disease associated with demyelination of central nervous system axons is a nutritional metabolic disorder.

In one embodiment, the nutritional metabolic disorder is vitamin B_{12} deficiency.

In another embodiment, the nutritional metabolic disorder is central pontine myelinolysis.

In one embodiment, the effective amount is an amount from 0.1 mg/kg to 400 mg/kg.

In a preferred embodiment, the effective amount is an amount from 0.1 mg/kg to 250 mg/kg.

In a further embodiment, the effective amount is an amount from 0.5 mg/kg to 400 mg/kg.

In another embodiment, the effective amount is an amount from 0.5 mg/kg to 300 mg/kg.

In another embodiment, the effective amount is an amount from 0.5 mg/kg to 250 mg/kg.

In further embodiment, the effective amount is an amount from 1.0 mg/kg to 250 mg/kg.

In another embodiment, the effective amount is an amount from 2.5 mg/kg to 225 mg/kg.

In yet another embodiment, the effective amount is an amount from 5.0 mg/kg to 200 mg/kg.

In a further embodiment, the effective amount is an amount from 10 mg/kg to 175 mg/kg.

In another embodiment, the effective amount is an amount from 25 mg/kg to 150 mg/kg.

In yet another embodiment, the dosage of antibodies against glatiramer acetate is an amount from 50 mg/kg to 125 mg/kg.

In a further embodiment, the effective amount is an amount from 75 mg/kg to 100 mg/kg.

The subject invention further provides a method of stimulating remyelination of central nervous system axons comprising contacting the axons with glatiramer acetate in an amount effective to stimulate remyelination of central nervous system axons.

The subject invention additionally concerns a method of treating a subject suffering from a disease associated with demyelination of central nervous system axons comprising administering to the subject glatiramer acetate in an amount effective to treat the disease associated with demyelination of central nervous system axons, wherein the disease associated with demyelination of central nervous system axons is selected from the group consisting of: acute disseminated

encephalomyelitis, transverse myelitis, demyelinating genetic diseases, spinal cord injury, virus-induced demyelination, Progressive Multifocal Leucoencephalopathy, HTLVI-associated myelopathy, and nutritional metabolic disorders.

The subject invention also contains a method of stimulating proliferation of lymphocytes comprising contacting the lymphocytes with an antibody directed against an epitope on glatiramer acetate in an amount effective to stimulate lymphocyte proliferation.

In this method, the antibody may be a humanized antibody directed against an epitope on glatiramer acetate.

In this method, the antibody may be not cross-reactive with MBP.

In this method, the antibody may consist essentially of IgG1.

In this method, the antibody may not react with mature oligodendrocytes.

In this method, the antibody may cross-react with SCH.

In this method, the antibody may primarily react with cells exhibiting a macrophage or microglial phenotype.

In this method, the antibody may be a monoclonal antibody.

In this method, the antibody may be a humanized polyclonal antibody directed against an epitope on glatiramer acetate.

In one embodiment, antibodies against glatiramer acetate are generated by intraperitoneal injection of glatiramer acetate into SJL mice. Alternatively, glatiramer acetate could be injected intradermally or intravenously. Other sources of

antibodies against glatiramer acetate are contemplated by the invention. These sources include, but are not limited to, other mice, rabbits, cats, goats, monkeys and humans.

Additionally contemplated by the present invention is a monoclonal antibody directed against an epitope on glatiramer acetate. These antibodies can be created by procedures known to those of skill in the art. Such procedures include, but are not limited to, the creation of hybridomas and antibody libraries.

A hybridoma is produced by the fusion of Normal B lymphocytes, which will not grow indefinitely in culture, and myeloma cells, which are immortal (11, 44). The selective medium most often used to culture such fused cells is called HAT medium, because it contains hypoxanthine, aminopterin, and thymidine (11). Normal B lymphocytes can grow in HAT medium, salvage mutants cannot, but their hybrids with Normal B lymphocytes can (11). Mutant myeloma cell lines that have lost the salvage pathways for purines (indicated by their inability to grow in HAT medium) are selected (11). These myeloma cells are then fused with normal B lymphocytes, creating hybridoma cells (11). Like myeloma cells, hybridoma cells can grow indefinitely in culture; like normal B lymphocytes, the fused cells have purine salvage-pathway enzymes and can grow in HAT medium (11). If a mixture of fused and unfused cells is placed in HAT medium, the unfused mutant myeloma cells and the unfused lymphocytes die, leaving a culture of immortal hybridoma cells, each of which produces a monoclonal antibody (11). Clones of hybridoma cells can be tested separately for the production of a desired antibody and the clones containing that antibody then can be cultured in large amounts (11).

Following the cloning of genes encoding antibodies, a library of filamentous phage can be prepared (26). Each phage has

the potential to display a unique antibody on its surface, which is the selectable phenotype (26). Within the phage coat is the genotype that encodes the displayed molecule. This linkage of displayed antibody phenotype with encapsulated genotype via the phage surface forms the basis of the technique (26). Typically, the antibody fragments are displayed on the surface of phage as either F_{ab} fragments, single-chain variable region fragments (scF_{vs}), or dimeric scF_{vs}, also known as diabodies, which differ from scF_{vs} in the reduced length of the linker peptide used and their preference to associate as dimers (26). Library construction is facilitated by the ready availability of phagemid vectors, which allow for construction and display of libraries of these antibody fragments using a single rare cutting restriction enzyme, SfiI (26). Selection of antibodies from the library is based on the displayed antibodies' binding specificity and affinity and is generally performed over several rounds of selection and amplification in a process known as panning (26).

Phage displayed antibody libraries can be screened by panning on purified antigens immobilized on artificial surfaces or by panning on cell surface expressed antigens (26). In contrast to panning on purified immobilized antigen, cell panning selects for antibodies that are more likely to bind to epitopes in vivo (26).

One method of improving the specificity of antibodies in an library is saturated mutagenesis of complementarity determining regions (CDR) (CDR walking mutagenesis) (26). In this approach, saturation mutagenesis of a CDR is constrained to libraries that examine all possible amino acids in the target CDR (26). Two strategies are employed -- either sequential or parallel optimization of CDR (26). In the sequential approach, the library of antibodies with a single randomized CDR is screened by several rounds of panning

against the antigen (26). The selected clone(s) are then used in the construction of a second library where a different single CDR is randomized (26). The panning, selection of clone(s) and construction of a library is repeated several times (26). Sequential optimization takes into account that optimal binding may result from the interdependence of CDRs (26). In the parallel approach, independent libraries are constructed where each library represents the randomization of given CDR (26). Each library is screened by several rounds of panning against the antigen (26). Then, the individually optimized CDRs are combined. If the free energy change of individually optimized CDRs combined is nearly equal to the sum of the free energy changes in the single optimized CDRs, the free energy changes are said to be additive (26). As additivity within the antibody binding site it virtually impossible to predict, Rader and Barbas believe that sequential CDR optimization is preferred over parallel (26).

The subject invention further contemplates humanized antibodies against glatiramer acetate. A humanized antibody is a non-human antibody which has been genetically engineered by the substitution of human nucleotide sequences in the nonvariable regions of the non-human antibodies (1, 40). Such substitutions reduce the immunogenicity of the antibodies in humans without significantly lowering the specificity of the antibodies.

One type of humanized antibody is a chimera, in which the variable region genes of a non-human antibody are cloned into a human expression vector containing the appropriate human light chain and heavy chain constant region genes (40). The resulting chimeric monoclonal antibody should have the antigen-binding capacity (from the variable region of the non-human source) and should be significantly less

immunogenic than the unaltered non-human monoclonal antibody.

Jones and his colleagues further humanized chimeric antibodies through a technique known as complementarity determining region (CDR) grafting (40). In this process, the antigen binding sites, which are formed by three CDRs of the heavy chain and three CDRs of the light chain, are excised from cells secreting non-human monoclonal antibodies and grafted into the DNA coding for the framework of the human antibody (40). Since only the antigen-binding site CDRs of the non-human antibody are transplanted, the resulting humanized antibody is less immunogenic than a chimeric antibody in which the entire variable domain is transplanted.

This process has been further improved by "reshaping," "hyperchimerization," and "veneering" (40). In the reshaping process on the basis of homology, the non-human variable region is compared with the consensus sequence of the protein sequence subgroup to which it belongs (40). Similarly, the selected human constant region accepting framework is compared with its family consensus sequence (40). The sequence analyses identify residues which may have undergone mutation during the affinity maturation procedure and may therefore be idiosyncratic (40). Inclusion of the more common human residues minimizes immunogenicity problems by replacing human acceptor idiosyncratic resides.

Hyperchimerization is an alternative method of identifying residues outside of the CDR regions that are likely to be involved in the reconstitution of binding activity (40). In this method, the human sequences are compared with non-human

variable region sequences and the one with highest homology is selected as the acceptor framework (40). As in the reshaping procedure, the "idiosyncratic" residues are replaced by the more commonly occurring human residues (40). The non-CDR residues that may be interacting with the CDR sequences are identified (40). Finally, one of these residues is selected to be included in the variable region framework (40).

"Veneering" is the process of replacing the displayed surfaces of proteins, or residues, which differ from those commonly found in human antibodies (40). Appropriate replacement of the outer residues may have little or no impact on the inner domains or interdomain framework (40). In the process of veneering, the most homologous human variable regions are selected and compared by each residue to the corresponding non-human variable regions (40). Then, the non-human framework residues, which differ from the human homologue, are replaced by the residues present in the human homologue (40).

The subject antibodies against glatiramer acetate can be administered by any method known to those of skill in the art. Such methods include, but are not limited to, intravenous, subcutaneous, intramuscular and intraperitoneal injection, and oral, nasal and rectal administration of the active substance and a pharmaceutically acceptable carrier.

In addition to in vivo methods of promoting remyelination, ex vivo methods of stimulating remyelination in CNS axons are encompassed by the present invention. For example,

antibodies against glatiramer acetate may be used in vitro to stimulate the proliferation and/or differentiation of glial cells, such as oligodendrocytes. These exogenous glial cells can then be introduced into the CNS using known techniques. Remyelination of CNS axons would be increased by raising the number of endogenous glial cells, as these cells play a critical role in the production of myelin.

In vitro methods of producing glial cells, or stimulating the proliferation of glial cells from mixed culture are also encompassed by the subject invention. For example, cells obtained from rat optic nerve, or rat brain, containing glial cells, are cultured as a mixed culture under conditions sufficient to promote growth of the cells. An effective amount of antibodies against glatiramer acetate is then added to the mixed culture and maintained under conditions sufficient for growth and proliferation of cells. The antibodies against glatiramer acetate stimulate the proliferation of glial cells in the mixed culture. Thus, the proliferation of glial cells cultured in the presence of antibodies against glatiramer acetate is increased, relative to the proliferation of glial cells grown in the absence of the antibodies.

The subject invention concerns treatment of a demyelinating central nervous system disease by a treatment regime that promotes production of a level of antibodies against glatiramer acetate or polyclonal B cell expansion that results in CNS remyelination.

In one embodiment, the treatment regime entails the

administration of glatiramer acetate. Regime variables could include, but not be limited to, dose, frequency of administration, sites of administration, and adjuvant co-administration. The appropriate regimen of treatment with glatiramer acetate should be determined empirically from patient studies.

Glatiramer acetate and antibodies against glatiramer acetate can be formulated into pharmaceutical compositions containing a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, adjuvants, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, sweeteners and the like. The pharmaceutically acceptable carriers may be prepared from a wide range of materials including, but not limited to, flavoring agents, sweetening agents and miscellaneous materials such as buffers and absorbents that may be needed in order to prepare a particular therapeutic composition. The use of such media and agents with pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated.

Glatiramer acetate and antibodies against glatiramer acetate can be formulated into any form known in the art using procedures available to one of skill in the art. In one embodiment, glatiramer acetate or an antibody against glatiramer acetate is introduced into the body by way of ingestion or inhalation. For example, they may be

administered by way of the mouth through feeding, through a stomach tube, by inhalation into the bronchial passages or by nasal inhalation. The composition contemplated by the subject invention may be administered either as a simple oral solution, as an emulsion or suspension formulation, as a solid oral dosage form (capsule or tablet), or even as a soft gelatin capsule. The present invention contemplates immediate release dosage forms and modified release dosage forms (including particulates, coated granules and pellets, emulsions, microemulsions and encapsulation in microspheres and nanospheres).

In one embodiment, the composition is formulated into a capsule or tablet using techniques available to one of skill in the art.

In another embodiment, glatiramer acetate or an antibody against glatiramer acetate is administered in another convenient form, such as an injectable solution or suspension, a spray solution or suspension, a rectal suppository, a lotion, a gum, a lozenge, a food or snack item. Food, snack, gum or lozenge items can include any ingestible ingredient, including sweeteners, flavorings, oils, starches, proteins, fruits or fruit extracts, vegetables or vegetable extracts, grains, animal fats or proteins. Thus, the present compositions can be formulated into cereals, snack items such as chips, bars, gum drops, chewable candies or slowly dissolving lozenges.

For both glatiramer acetate and antibodies against glatiramer acetate, one of skill in the art can readily substitute

structurally-related amino acids without deviating from the spirit of the invention. The present invention includes polypeptides and peptides which contain amino acids that are structurally related to tyrosine, glutamic acid, alanine or lysine and possess the ability to stimulate the production of polyclonal antibodies against them. Such substitutions retain substantially equivalent biological activity in their ability to suppress or alleviate the symptoms of the CNS disease. These substitutions are structurally-related amino acid substitutions, including those amino acids which have about the same charge, hydrophobicity and size as tyrosine, glutamic acid, alanine or lysine. For example lysine is structurally-related to arginine and histidine; glutamic acid is structurally-related to aspartic acid; tyrosine is structurally-related to serine, threonine, phenylalanine and tryptophan; and alanine is structurally-related to valine, leucine and isoleucine. These and other conservative substitutions, such as structurally-related synthetic amino acids, are contemplated by the present invention.

Moreover, glatiramer acetate can be composed of l-or d- amino acids. As is known by one of skill in the art, l-amino acids occur in most natural proteins. However, d- amino acids are commercially available and can be substituted for some or all of the amino acids used to make glatiramer acetate. The present invention contemplates glatiramer acetate consisting essentially of l-amino acids, as well as glatiramer acetate consisting essentially of d-amino acids.

Experimental Details

EXPERIMENTAL METHODS

Mice

All mice used in the experiments were SJL/J mice.

Virus

The Daniel's strain of Theiler's murine encephalomyelitis virus (TMEV) was used in all experiments. The original virus stock was obtained from J. Lehrich and B. Arnason after eight passages in cultured baby hamster kidney (BHK) cells (17). The virus was passaged an additional six times at a multiplicity of infection of 0.1 plaque-forming units per cell. Cell-associated virus was released by freeze-thawing and sonication. The lysate was clarified by centrifugation and stored in aliquots at -70°C.

Glatiramer Acetate Injections

All injections of glatiramer acetate, alone or in IFA, were given subcutaneously.

Antibody Injections

All antibodies were dissolved in PBS and administered intraperitoneally. Normal antibodies were protein G-purified antibodies isolated from commercially purchased mouse serum. These were used as a control for antibodies against glatiramer acetate.

Spinal Cord Lesions: Demyelination and Remyelination Quantitation

Areas of spinal cord demyelination and remyelination were determined from multiple cross-sections of plastic-embedded spinal cords, using a camera lucida system and a computerized drawing tablet (20). Briefly, mice were sacrificed and by

perfused by intracardiac puncture with Trumps fixative, containing 4% formaldehyde and 1% glutaraldehyde. Spinal cords were removed, post-fixed in osmium textroxide, and sectioned into 1-mm blocks. Every third block (10-12 blocks per spinal cord; cervical to lumbar range) was embedded in Araldite plastic. The embedded tissues were cross-sectioned, and the slides stained with 4% paraphenylenediamine to highlight the myelin sheaths.

Using a camera lucida attached to a Zeiss photomicroscope and a ZIDAS interactive digital analysis system, three parameters were measured from each slide: total white matter area, demyelinated lesion area, and remyelination area. Outline of these regions were traced and the areas calculated by the computerized digital analysis system. Demyelination was expressed as the total lesion area as a percentage of total white matter area. Remyelination was expressed as the total remyelination area as a percentage of the total demyelinated lesion area. The criterion for remyelination by oligodendrocytes was abnormally thin myelin sheaths. All remyelination data refers to oligodendrocyte-mediated remyelination. Occasionally, Schwann-cell mediated remyelination was observed, characterized by abnormally thick myelin sheaths and nuclei juxtaposed to the myelin sheath.

Brain Pathology Scoring

Mice were sacrificed and perfused by intracardiac puncture with Trumps fixative, containing 4% formaldehyde and 1% glutaraldehyde. Brains were removed and post-fixed in Trumps. Each brain was sectioned coronally into three pieces by cuts through the infundibulum and optic chiasm. The

pieces were then dehydrate and embedded in paraffin. Sections from each block were mounted on slides and stained with hematoxylin and eosin to identify pathology in the following brain regions: cortex, corpus callosum, hippocampus, brainstem, striatum, and cerebellum. Pathologic scores were assigned without knowledge of the experimental treatment. Each area of the brain was graded as follows:

0 = no inflammation

1 = minimal inflammation, confined to perivasculature

2 = moderate inflammation, including parenchyma infiltration, but no tissue damage

3 = intense parenchyma inflammation with minor but definite tissue damage (loss of tissue architecture, cell death, neurophagia, neuronal vacuolation)

4 = extensive inflammation and tissue damage.

ELISA

An indirect ELISA was performed in which serum or a purified antibody was applied to plates that were pre-coated with glatiramer acetate or other protein antigens. The antigen of interest was dissolved in 0.1 M carbonate buffer, pH 9.5, and applied at 1 ug per well in 96-well polystrene plates. Incubation was overnight at 4°C. Plates were then rinsed with PBST (phosphate buffered saline containing 0.05% Tween 20 detergent) and incubated for 1 hr in PBSM (phosphate buffered saline containing 5% defatted milk powder). Plates were rinsed with PBST, then incubated for 4 hr at room temperature with 50X PBS. Plates were rinsed with PBST, then incubated with biotinylated secondary antibodies that were raised in goat. The secondary antibodies, diluted in PBSM 1:50, were specific to mouse IgG or mouse IgM. Incubation was for 2 hr

at room temperature. Plates were rinsed in PBST, then PBS. Streptavidin-alkaline phosphatase conjugate diluted in PBS was applied to the plates for 2 hr room temperature. Plates were rinsed with PBST, with a final rinse in water. The colored reaction product was produced by incubation with p-nitrophenyl phosphatase in 0.1 M carbonate buffer plus 1 mM magnesium chloride. The reaction was stopped with 0.5 N sodium hydroxide. Optical absorbency was measured at a wavelength of 405 nm.

Immunohistochemistry on Cultured Cells

Immunohistochemistry was performed on the following cultured cells: CNS glia (mixed or oligodendrocyte-enriched) derived from neonatal rat brains, CNS glia derived from adult human brain biopsies (obtained from surgical correction of epilepsy), and mouse peritoneal macrophages obtained from 5-day thioglycollate-stimulated peritoneum and grown in culture for 1-3 weeks.

Application of primary antibodies in PBS buffer was performed with ice-cold solutions with culture plate on ice with the intention of staining the cell surface. Primary antibodies were applied for 30-45 min. After rinsing in PBS for 10 min, fluorophore-conjugated secondary antibodies diluted in ice-cold PBS were applied for 30 min. Cells were then rinsed with PBS for 10-15 min. Fixation with 4% paraformaldehyde occurred either once, following the final PBS rinse, or twice, just prior to secondary antibody application and following the final PBS rinse. Cells were viewed with Olympus™ fluorescent microscopes.

The primary antibodies included antibodies against glatiramer acetate, 4-40 ug/ml, Normal antibodies, 20 ug/ml, anti-GFAP (astrocyte marker), 01, 04, A2B5, 94.03 (oligodendrocyte markers), isolectin B4, CD11b (complement receptor 3)(activated microglia and macrophage markers). The secondary antibodies were anti-species IgG or IgM, raised in goat, and fluorophore-conjugated.

Purification of Antibodies Against Glatiramer Acetate in SJL/J Mice (Figure 25)

The steps involved in purification of antibodies against glatiramer acetate were:

1. *Immunization* of SJL/J mice. Mice were immunized by eight, subcutaneous injections of glatiramer acetate + IFA (0.1 mg/injection between Days 0-50).
2. *Serum isolation*. Mice were bled 9 times between Days 14-56 from the time of first immunization. After each bleed, blood was stored overnight at 4°C, then centrifuged to isolate serum. Serum was stored at -20°C until all bleeds were completed.
3. *Affinity column isolation of antibodies against glatiramer acetate*. Glatiramer acetate was coupled to NHS-activated Hi-Trap affinity columns (Amersham Pharmacia) by the manufacturer's recommended procedures. Serum was thawed, pooled, centrifuged to remove serum lipid, diluted with phosphate buffer, and run over the glatiramer acetate affinity column. After rinsing the column, antibodies against glatiramer acetate were eluted with glycine-HCl (pH 3.0) into Tris buffer (pH 8.0). The OD_{260} of the eluted samples was determined as a measure of protein content. Most of the antibodies

against glatiramer acetate were eluted in the first 2 fractions.

4. *Dialysis*. Eluted fractions containing high levels of antibodies against glatiramer acetate were pooled and dialyzed in PBS.

5. *Purity and protein analysis*. Dialyzed antibodies against glatiramer acetate were analyzed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and found to be almost exclusively comprised of product representative of antibody heavy and light chains. Bicichoninic acid protein assay was used to determine the total protein content of the sample, using bovine serum albumin (BSA) as the standard. The determined concentration of 4.8 mg/ml was considered as the concentration of antibodies against glatiramer acetate.

6. *Storage*. Dialyzed antibodies against glatiramer acetate were filter-sterilized with a 0.22 μm filter and stored at 4°C. Over 2 years of storage, no precipitate or contamination was observed and glatiramer acetate reactivity by ELISA and cellular binding was retained.

Purification of Normal Antibodies (Figure 26)

The steps involved in purification of Normal antibodies were:

1. *Protein G column isolation of antibodies against glatiramer acetate*. Normal mouse serum (SIGMA™ commercial preparation) was centrifuged to remove serum lipid, diluted with phosphate buffer, and run over a Protein G column (Hi-trap Protein G column; Amersham Pharmacia). After rinsing the column, Normal antibodies were determined as a measure of protein content.

2. *Dialysis*. Eluted fractions containing high levels of antibodies were pooled and dialyzed in PBS.

3. *Purity and protein analysis*. Dialyzed Normal antibodies were analyzed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and found to be almost exclusively comprised of product representative of antibody heavy and light chains. Bicichoninic acid protein assay was used to determine the total protein content of the sample, using BSA as the standard. The determined concentration of 2.6 mg/ml was considered as the Normal antibody concentration.

4. *Storage*. Dialyzed Normal antibodies were filter-sterilized with a 0.22 μm filter and stored at 4°C. Over 2 years of storage, no precipitate or contamination was observed.

EXAMPLE 1: EFFECT OF GLATIRAMER ACETATE TREATMENT ON EXTENT OF SPINAL CORD WHITE MATTER PATHOLOGY DURING EARLY DISEASE.

Procedure

Mice were injected with glatiramer acetate (0.1 mg/injection) in IFA or IFA alone on Day -15 and Day 7, relative to virus injection. Additional injections of either glatiramer acetate alone or phosphate buffered saline (PBS) were performed on Days -7, 0, 13, 21, 32, and 41. Virus was injected on Day 0. Mice were sacrificed on Day 45 post-infection (60 days of glatiramer acetate treatment), and spinal cord demyelinating pathology was measured.

Results

Glatiramer acetate exerted no statistically significant

effect on the extent of demyelination by 45 days post-infection (Figure 1). However, a trend towards reduced demyelination was present (T-test, P=0.08).

EXAMPLE 2: EFFECT OF GLATIRAMER ACETATE TREATMENT DURING LATE DISEASE

Experiment 2A: Effect of Glatiramer Acetate on the Extent of Spinal Cord White Matter Pathology During Late Disease

Procedure

Chronically infected mice (124-349 days post -infection) were treated subcutaneously for periods of 41-76 days. One group of mice received 0.1 mg glatiramer acetate by 8 injections of 0.1 mg each. These mice were sacrificed after 65 days. A second group of mice received 0.1 mg glatiramer acetate/IFA by 4-8 injections at equal intervals, 0.1 mg/injection. The members of this group were sacrificed after 65-76 days. A third group of mice received 5 mg glatiramer acetate/IFA by 1-2 injections each of 5 mg. Sacrifice of these mice occurred after 41-65 days. In all groups, spinal cord demyelination was measured after sacrifice.

Results

Treatments with the high dose of glatiramer acetate caused an expansion of the demyelinating lesions ($P < 0.05$, compared to PBS treated mice, unpaired t-test) (Figure 2). Low doses of glatiramer acetate, either alone or in combination with adjuvant, did not alter the extent of demyelination.

Experiment 2B: Effect of Glatiramer Acetate on Extent of Remyelination in the Spinal Cord During Late Disease

Procedure

Chronically infected mice (124-349 days post-infection) were treated subcutaneously with glatiramer acetate as in Experiment 2A. After sacrifice, spinal cord remyelination was measured in all mice. Spinal cords with less that 4.0% demyelination were excluded from the remyelination analysis (2 from PBS, 1 from IFA and 2 from 0.1 mg glatiramer acetate).

Results

Glatiramer acetate treatment did not affect the extent of remyelination during late disease (Figure 3). IFA enhanced remyelination ($p < 0.05$, compared to PBS, unpaired t-test). The beneficial effects of IFA raises the possibility that polyclonal B cell activation with appropriate stimulants might promote remyelination.

Experiment 2C: Effect of Glatiramer Acetate on Brain Pathology During Late Disease

Procedure

Chronically infected mice (250 days post-infection) were treated by subcutaneous injection with either PBS, IFA or 1.0 mg glatiramer acetate with IFA, twice weekly for a total of 8 injections. After 29 days of treatment, mice were sacrificed and paraffin-embedded brain sections were scored for the extent of pathology, as explained in the Experimental Methods section.

Results

Figure 4 shows the effect of glatiramer acetate on brain pathology. Each symbol is the score from an individual animal. Glatiramer acetate treatment did not affect the extent of the brain pathology during late disease.

Experiment 2D: Effect of Antibodies Against Glatiramer Acetate on Demyelination During Late Disease

Procedure

Chronically infected mice (160-477 days post-infection) were treated by intraperitoneal injection with antibodies for periods of 36-76 days. One group of mice received Normal antibodies, administered in 10 injections of 0.05 mg each for a total of 0.5 mg over 42 days. A second group of mice received 0.5 mg antibodies against glatiramer acetate, administered in 10 injections of 0.05 mg each, for a total of 0.5 mg over 41 - 42 days. The final group of mice received 1.5 mg antibodies against glatiramer acetate, administered in 5 injections of 0.3 mg each for a total of 1.5 mg over 36 days. Spinal cord white matter demyelinating pathology was measured post-sacrifice for all mice.

Results

Neither antibodies against glatiramer acetate nor Normal antibodies, when passively transferred, affected the extent of demyelinating pathology during late disease (Figure 5).

Experiment 2E: Effect of Antibodies Against Glatiramer Acetate on Remyelination During Late Disease

Procedure

Chronically infected mice (160-477 days post infection) were treated by antibodies, following the procedure of Experiment 2D. After sacrifice, spinal cord remyelination was measured. Spinal cords with less that 4.0% demyelination were excluded from the remyelination analysis (2 from PBS, 2 from 0.5 mg antibodies against glatiramer acetate, and 1 from 1.5 mg antibodies against glatiramer acetate.

Results

Glatiramer acetate antibody treatment at both doses increased the extent of remyelination ($P < 0.05$ for both compared to PBS, unpaired t-tests) (Figure 6). Normal antibodies did not affect remyelination, suggesting that the beneficial effect of antibodies against glatiramer acetate was through specific antigen (epitope) interactions rather than through nonspecific interactions of antibody heavy chains with F_c receptors.

EXAMPLE 3: SERUM TITRES OF GLATIRAMER ACETATE IgG

Experiment 3A: Serum Titres of Glatiramer Acetate IgG in Non-infected, Immunized Mice

Procedure

Non-infected mice were immunized with glatiramer acetate/IFA on Days 0, 4, 8, 15, and 26 (0.1 mg glatiramer acetate/injection) or on Days 0, 3, 7, 10, and 21 (1.0

mg/injection). On Days 0, 7, 14, 19, 28, 33 and 51, blood was collected from 26-35 mice, serum was isolated and pooled. ELISA was performed using glatiramer acetate-coated plates and biotinylated anti-mouse IgG as the secondary antibody.

Results

Shown in Figure 7 are the 1:1000 sera dilutions. Serum titres of Cop 1 IgG were first detectable 14 days post-immunization and increased over time. Approximately 3 weeks were required to achieve high antibody titres. Varying the glatiramer acetate doses from 0.1 - 1.0 mg and altering the timing of injections did not significantly influence glatiramer acetate IgG titres, although the immunization and sampling regimens also differed. These data helped to determine how long infected mice should be treated with glatiramer acetate in order to evaluate whether antibodies against glatiramer acetate generated by immunization of infected mice can promote remyelination.

Experiment 3B: Serum Titres of Glatiramer Acetate IgG in Chronically Diseased Mice After Immunization

Procedure

Mice infected for 124 days were immunized with glatiramer acetate or glatiramer acetate/IFA at 0.1 mg/injection for a total for 0.8 mg from Days 0-50. Mice were sacrificed on Day 65 post-immunization. Then, serum was isolated and ELISA was performed on glatiramer acetate-coated plates. The secondary antibody was biotinylated anti-mouse IgG.

Results

There was significant variability in the production of antibodies against glatiramer acetate among individual mice immunized with glatiramer acetate alone (Figure 8). In contrast, antibody levels were more similar among mice treated with glatiramer acetate and IFA. Immunization with glatiramer acetate and IFA generated much higher antibody levels than immunization with glatiramer acetate alone. No glatiramer acetate IgG was detected in serum from mice treated with either PBS or IFA alone.

Experiment 3C: Serum Titres of Glatiramer Acetate IgG in Chronically Diseased Mice

Procedure

Chronically diseased mice, infected for 196-286 days, were immunized with glatiramer acetate. The first group of mice received 5 mg glatiramer acetate/IFA injections on Days 0 and 20. Serum was isolated from these mice on Day 62. The next group of mice received 5 mg glatiramer acetate/IFA injections on Day 0. Serum was isolated from these mice on Day 41. The last group of mice received 0.1 mg glatiramer acetate/IFA injections on Days 0,25,37, and 64. Serum was isolated on Day 76. For all groups, ELISA was performed using glatiramer acetate-coated plates. The secondary antibody was biotinylated anti-mouse IgG.

Results

The production of antibodies against glatiramer acetate was highly dose-dependent (Figure 9). Very high glatiramer acetate IgG titres were reached after immunization and a

single boost using 5 mg glatiramer acetate injections. The titres were much higher than following 4 injections of 0.1 mg glatiramer acetate. However, relatively high titres were reached even using 0.1 mg glatiramer acetate over time. Individual symbols are the means (± SEM) for 4-5 mice, except for the untreated group (2 mice).

Experiment 3D: Serum Titres of Glatiramer Acetate IgG Following Treatment with Passively Transferred Antibodies Against Glatiramer Acetate vs. Glatiramer Acetate Immunization

Procedure

One group of chronically diseased mice (196-348 days of infection) received 5 mg glatiramer acetate in IFA in a single subcutaneous injection. The other group of chronically diseased mice received 10 x 50 µg intraperitoneal injections of antibodies against glatiramer acetate, twice weekly. The final passive transfer was on Day 35. After 41 days of treatment, both groups of mice were sacrificed and serum titres of Copolymer IgG was measured by ELISA. The secondary antibody was biotinylated anti-mouse IgG.

Results

Glatiramer acetate IgG was detected in serum 6 days following the final passive transfer of antibodies against glatiramer acetate, but levels were much lower than in mice that received a single immunization with high dose glatiramer acetate (Figure 10). Since IgG is cleared with a half-life of approximately 3 weeks, the low serum titre of antibodies against glatiramer acetate 6 days after the final passive

transfer suggests that low levels of antibodies against glatiramer acetate are sufficient for promotion of remyelination.

EXAMPLE 4: REACTIVITY OF PURIFIED ANTIBODIES AGAINST GLATIRAMER ACETATE AND PURIFIED NORMAL ANTIBODIES

Experiment 4A: Glatiramer Acetate Reactivity of Purified Antibodies Against Glatiramer Acetate and Purified Normal Antibodies: IgG and IgM

Procedure

The procedures for isolating antibodies against glatiramer acetate and Normal IgG were as described in Experimental Methods. Purified antibodies against glatiramer acetate or Normal antibodies were assayed by ELISA using glatiramer acetate-coated plates. The secondary detection antibodies were either biotinylated anti-mouse IgG or biotinylated anti-mouse IgM.

Results

Antibodies against glatiramer acetate had high reactivity to glatiramer acetate (Figure 11). Both glatiramer acetate IgG and glatiramer acetate IgM were detected. Normal antibodies had little or no IgG- or IgM-reactivity to glatiramer acetate.

Experiment 4B: Protein Polyreactivity of Purified Antibodies Against Glatiramer Acetate and Purified Normal Antibodies

Procedure

Proteins were adsorbed to ELISA plates as described in Experimental Methods. Plates were then reacted with antibodies against glatiramer acetate, Normal antibodies, or SCH 94.03 monoclonal IgM antibodies. Secondary antibodies were anti-mouse IgG or anti-mouse IgM.

Results

Very low polyreactivity of glatiramer acetate IgG/IgM and Normal IgG was observed (Figure 12). The only significant cross-reactivity was to rabbit myosin heavy chain. This contrasts with multiple cross-reactivities seen for SCH 94.03 IgM, as reported in U.S. Patent 5,591,629 (1). This supports the hypothesis that the mechanism by which antibodies against glatiramer acetate promote remyelination is different from the mechanism used by polyreactive IgM antibodies such as SCH 94.03.

EXAMPLE 5: ISOTYPE ANALYSIS OF ANTIBODIES AGAINST GLATIRAMER ACETATE AND NORMAL ANTIBODIES

Procedure

Purified antibodies against glatiramer acetate and Normal antibodies were isotyped by ELISA using an antibody isotyping kit (Pierce).

Results

All tested isotypes were found in both glatiramer acetate

antibodies and Normal antibodies (Figure 13). The glatiramer acetate antibody preparation had a higher relative level of IgG1 than other isotypes, compared to the IgG1 level in Normal antibodies. IgG1 (non-complement fixing in mice) appears to be the most abundant in the antibodies against glatiramer acetate. The apparent abundance of IgG1 implies that complement activation would not be a significant consequence of glatiramer acetate antibody treatment.

EXAMPLE 6: EFFECT OF ANTIBODIES AGAINST GLATIRAMER ACETATE ON IN VITRO PROLIFERATION OF LYMPH NODE-DERIVED LYMPHOCYTES FROM MBP $_{84-102}$- AND PLP $_{179-191}$-IMMUNIZED MICE

Experiment 6A: Antibodies Against Glatiramer Acetate Stimulate in Vitro Proliferation of Lymph Node-derived Lymphocytes from Mice

Procedure

Two mice were subcutaneously injected in the flanks with MBP $_{84-102}$ in complete Fruend's adjuvant (CFA). MBP $_{84-102}$ was obtained by following the procedure of Hawes et al (14). A method similar to that of Tuohy et al. was employed to produce PLP $_{179-191}$ (38). PLP $_{179-191}$ in CFA was subcutaneously injected into the flanks of 1 mouse. After 10 days, inguinal and per-aortic lymph nodes were removed, dissociated, and grown for 53 hours in culture in medium alone or in the presence of MBP $_{84-102}$, PLP $_{179-191}$, Normal antibodies, antibodies against glatiramer acetate or glatiramer acetate. In the last 13 hours of culture, 1 μCi of [^{3}H]-thymidine was added and its incorporation measured by scintillation counting of harvested cells.

Results

Antibodies against glatiramer acetate (25 μg/ml) stimulated lymphocyte proliferation to a comparable level as 50 μg/ml of specific peptide (stimulation indices = 12-17) (Figure 14). Normal antibody-induced proliferation occurred, but to a much lower level than that induced by antibodies against glatiramer acetate or a specific peptide, suggesting that antigen-specificity of antibodies against glatiramer acetate contributed to its effect. Glatiramer acetate did not induce significant proliferation. The stimulation of proliferation is consistent with an effect of antibodies against glatiramer acetate on antigen presentation, but other explanations, such as direct binding to lymphocytes, are also possible.

Experiment 6B: Antibodies Against Glatiramer Acetate Stimulate in Vitro Proliferation of Lymph Node-derived Lymphocytes from MBP_{84-102}-immunized Mice

Procedure

Mice were subcutaneously injected in the flanks with MBP_{84-102} (2 mice) peptide in CFA. After 10 days, inguinal and peri-aortic lymph nodes were removed, dissociated, and grown for 74 hours in cultures in medium alone or with additional additives as shown in Figure 15. In the last 12 hours of culture, 1 μCi of [^{3}H]-thymidine was added and its incorporation measured by scintillation counting of harvested cells.

Results

Antibodies against glatiramer acetate (25 ug/ml) stimulated

lymphocyte proliferation (stimulation indices = 6 and 13) (Figure 15). Normal antibodies did not induce proliferation at a comparable antibody concentration. As in Experiment 6A, the stimulation of proliferation could be attributed to the effect of antibodies against glatiramer acetate on antigen presentation, but there are other viable theories, such as direct binding to lymphocytes.

Experiment 6C: Antibodies Against Glatiramer Acetate Do Not Stimulate in Vitro Proliferation of Lymph Node-derived Lymphocytes from Non-immunized Mice

Procedure

Inguinal and peri-aortic lymph nodes from non-immunized SJL/J mice were removed, dissociated, and grown for 74 hours in culture in medium alone or or in the presence of MBP $_{84-102}$, PLP $_{179-191}$, Normal antibodies, antibodies against glatiramer acetate or glatiramer acetate. In the last 12 hours of culture, 1 μCi of [^{3}H]-thymidine was added and its incorporation measured by scintillation counting of harvested cells.

Results

Figure 16 shows that no treatment, including antibodies against glatiramer acetate (25 μg/ml), stimulated lymphocyte proliferation to a significant degree over the baseline level (medium alone). This suggests that antibodies against glatiramer acetate stimulate lymphocyte proliferation (Figures 14 and 15) only during an active immune response to myelin peptides or other antigens.

EXAMPLE 7: GLATIRAMER ACETATE ANTIBODY BINDING TO CULTURED CELLS

Experiment 7A: Copolymer 1 Antibody Binding to Cultured Cells from the CNS

Procedure

The methodology is described above in the Experimental Methods. Briefly, all staining was performed with ice-cold solutions, with the culture plate on ice, and prior to fixation in order to bind the cell surface. The primary antibodies for these experiments included antibodies against glatiramer acetate, 4-40 μg/ml, Normal antibodies, 20 μg/ml, anti-GFAP (astrocyte markers), 01, 04, A2B5, 94.03 (oligodendrocyte markers), isolectin B_4, CD11b (complement receptor 3) (activated microglia and macrophage markers). The secondary antibodies were directed against IgG or IgM of the appropriate species.

Results

CNS glial cultures derived from neonatal rat brains

Glatiramer acetate IgG and IgM (secondary antibodies were isotype-specific) stained a small population of cells that were distinct from oligodendrocytes. Mature oligodendrocytes were readily identified by elaborate process extension and by staining with oligodendrocyte markers. In contrast, glatiramer acetate antibody-positive cells did not have elaborate process extension. Rather, they had the phenotype of activated microglia. No co-labeling of cells was observed with antibodies against glatiramer acetate or any of the

oligodendrocyte markers, whereas cells were co-labeled with glatiramer acetate IgG and the activated microglia markers, isolectin B_4 or Mac-1.

Stronger staining by antibodies against glatiramer acetate was observed after using secondary antibodies directed against mouse IgG than against mouse IgM. Immunostaining with antibodies against glatiramer acetate exceeded the staining by Normal IgG and by secondary antibodies alone, suggesting that antibodies against glatiramer acetate recognized specific cell-surface epitopes rather than being bound simply by F_c receptors. Very little immunostaining of GFAP (an intracellular antigen) was observed, suggesting that the staining seen with other antibodies (i.e., antibodies against glatiramer acetate) was against cell surface antigens.

Human Mixed Glial Cultures (Figure 18)

In CNS glial cell cultures, a population of cells stained positive for glatiramer acetate IgG. These cells were similar in phenotype to those in rat glial cultures and distinct from the O4-positive oligodendrocytes, which had elaborate process extension. These data are consistent with glatiramer acetate IgG staining of activated microglia, rather than oligodendrocytes.

Experiment 7B: Binding to Cultured Mouse Peritoneal Macrophages by Antibodies Against Glatiramer Acetate

Procedure

The methodology is described above in the Experimental

Methods. Briefly, peritoneal macrophages (similar in phenotype and function as activated microglia) were derived from the peritoneum of SJL mice, 5 days after intraperitoneal stimulation with 3% sterile thioglycollate broth. Cells were then cultured for 1-3 weeks prior to staining. Antibodies were diluted in ice-cold PBS and the solutions applied to culture plates on ice in order to detect cell surface staining. The primary antibody incubations consisted of combinations of the following: antibodies against glatiramer acetate, 40 μg/ml, Normal antibodies, 20 μg/ml, isolectin B_4, CD11b (complement receptor 3) (activated microglia and macrophage markers), glatiramer acetate, 80-200 μg/ml.

Results

Figure 17 demonstrates that incubation with antibodies against glatiramer acetate under cold, unfixed conditions resulted in IgG staining of a subset of cells. Acetone fixation/permeabilization prior to incubation with antibodies against glatiramer acetate resulted in staining of all cells, suggesting that antibodies against glatiramer acetate were highly reactive to intracellular antigens. Normal antibodies did not stain acetone-fixed cells. Fixation with 4% paraformaldehyde prior to incubation with antibodies against glatiramer acetate did not significantly alter the staining pattern as compared to fixation following incubation with antibodies against glatiramer acetate. Glatiramer acetate IgG-positive cells always co-stained with isolectin B_4 and Mac-1. Four-hour pre-incubation of macrophages at 37°C with a variety of agents (20 μg/ml glatiramer acetate, myelin homogenate, kidney homogenate, MBP $_{84-102}$, PLP $_{179-191}$) did not influence subsequent staining by glatiramer acetate IgG,

compared to cells preincubated only with medium. This suggests that MHC Class II presentation of processed glatiramer acetate or other antigens at the cell surface did not influence the binding of antibodies against glatiramer acetate. Co-incubation of antibodies against glatiramer acetate with glatiramer acetate and macrophages greatly increased the intensity of glatiramer acetate IgG staining and the number of cells stained, suggesting that antibodies against glatiramer acetate complexed with glatiramer acetate bound much more extensively to macrophages than antibodies against glatiramer acetate alone. Co-incubation of antibodies against glatiramer acetate with myelin homogenate, SCH, or kidney homogenate did not alter glatiramer acetate staining. Punctate staining was present only when antibodies against glatiramer acetate were co-incubated with glatiramer acetate. This staining pattern was indicative of clustered receptors, suggesting that MHC Class II molecules may have been bound by glatiramer acetate.

Discussion

By morphological and co-immunolabeling criteria, antibodies against glatiramer acetate bound to subpopulations of activated microglia and macrophages in culture. This staining pattern contrasts with that of monoclonal antibody SCH 94.03 and other remyelination-promoting antibodies, which bind preferentially to the surface of oligodendrocytes (1). Glatiramer acetate IgG staining was stronger than glatiramer acetate IgM staining. Antibodies against glatiramer acetate bound both surface and intracellular antigens. Staining of antibodies against glatiramer acetate was greatly increased by co-incubation with glatiramer acetate, but not by pre-

incubation of macrophages with glatiramer acetate.

The increased staining following co-incubation with glatiramer acetate might reflect binding of glatiramer acetate: antibody complexes to MHC Class II molecules, which are known to bind glatiramer acetate (12, 37). In addition, the binding to microglia/macrophages in vivo might modulate cellular function, thereby triggering a more permissive environment for remyelination.

EXAMPLE 8: LESION PATHOLOGY AND REMYELINATION

Procedure

Chronically infected mice (6 months or longer post-infection) were treated with PBS, glatiramer acetate (0.1 mg/injection), or antibodies against glatiramer acetate (1.5 mg total).

Results

PBS Treatment

Mice treated with PBS showed extensive demyelination and macrophage filtration of lesions. Remyelination, characterized by abnormally thin myelin sheaths, was virtually absent (Figures 19 - 20).

Glatiramer Acetate Treatment

Lesions were extensively demyelinated and infiltrated with macrophages in glatiramer acetate-treated mice (Figures 21 - 22). Patches of significant remyelination were occasionally observed in some lesions, but quantitively, the remyelination is not more extensive than following the PBS treatment.

Treatment with Antibodies Against Glatiramer Acetate

The lesions of mice treated with antibodies against glatiramer acetate showed extensive oligodendrocyte-mediated remyelination (Figures 23 - 24).

Discussion

Glatiramer acetate has proved to be effective in treating multiple sclerosis (2 - 6, 16). No prior studies have proposed that glatiramer acetate works by inducing the production of polyclonal antibodies against glatiramer acetate. Instead, scientists have theorized that glatiramer acetate disrupts the MHC/TCR complex formation to specific peptides (25), induces glatiramer acetate-specific suppressor cells in vivo (7) or binds directly to major histocompatibility complex class II to replace MBP peptides (5, 37).

Without being limited to any specific mechanism, one hypothesis regarding the mechanism of glatiramer acetate in the treatment of CNS diseases is that it actively induces a protective humoral immune response. Preliminary data indicate that patients treated with glatiramer acetate develop very high antibody titers to glatiramer acetate. There seems to be a strong positive correlation between the presence of antibodies against glatiramer acetate and therapeutic efficacy.

One theory concerning the way in which antibodies promote remyelination is that they directly stimulate oligodendrocyte progenitor proliferation, migration, or differentiation. It is possible to promote remyelination only after approximately

4 months of infection, a time at which most of the active myelin ingestion appears to have subsided. Treatments at earlier than 4 months of infection have not promoted remyelination, suggesting that lesions reach a state of "maturation", which poises them for repair.

However, antibodies against glatiramer acetate appear to promote remyelination through an immunomodulatory mechanism, rather than through direct stimulation of oligodendrocyte differentiation. The proposed mechanism does not limit the subject invention. This hypothesis is supported by numerous findings of the subject invention. For example, in human glial cell cultures, antibodies against glatiramer acetate appeared to bind microglia by morphological criteria and by the criteria of co-immunostaining with activated microglia markers. The glatiramer acetate-positive cells were distinct from 04-positive oligodendrocytes, which were extensively arborized. In addition, antibodies against glatiramer acetate stimulated lymphocyte proliferation in vitro. Antibodies against glatiramer acetate also had very low polyreactivity to other proteins, unlike other remyelination-promoting antibodies (e.g., SCH 94.03 monoclonal antibody), which cross-react with many protein antigens (1). Furthermore, antibodies against glatiramer acetate comprised a spectrum of antibody isotypes, unlike the predominance of IgMs in other remyelination-promoting antibodies (1).

The subject invention demonstrates that antibodies against glatiramer acetate promote repair of demyelinated lesions of the spinal cord by inducing remyelination. It appears that antibodies against glatiramer acetate bind to activated

microglia, macrophages, and possibly to other similar types of cells, such as dendritic cells. Binding to these cells may modulate their functions, thereby facilitating remyelination by oligodendrocytes. Potential effects of the binding of antibodies against glatiramer acetateinclude alteration in antigen presentation, lymphocyte proliferation, and cytokine/growth factor production.

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What is claimed is:

1. A humanized antibody directed against an epitope on glatiramer acetate (Copolymer 1).

2. The antibody of claim 1, wherein the antibody is not cross-reactive with myelin basic protein (MBP).

3. The antibody of claim 1, wherein the antibody consists essentially of IgG1.

4. The antibody of claim 1, wherein the antibody does not react with mature oligodendrocytes.

5. The antibody of claim 1, wherein the antibody cross-reacts with spinal cord homogenate (SCH).

6. The antibody of claim 1, wherein the antibody primarily reacts with cells exhibiting a macrophage or microglial phenotype.

7. The antibody of claim 1, wherein the antibody is a monoclonal antibody.

8. The antibody of claim 1, wherein the antibody is a polyclonal antibody.

9. A F_{ab} fragment that binds to an epitope on glatiramer acetate (Copolymer 1).

10. A pharmaceutical composition comprising an antibody

directed against an epitope on glatiramer acetate (Copolymer 1) in an amount effective to treat a disease associated with demyelination of central nervous system axons and a pharmaceutically acceptable carrier.

11. The pharmaceutical composition of claim 10, wherein the antibody is a humanized antibody.

12. The pharmaceutical composition of claim 10, wherein the antibody is not cross-reactive with myelin basic protein (MBP).

13. The pharmaceutical composition of claim 10, wherein the antibody consists essentially of IgG1.

14. The pharmaceutical composition of claim 10, wherein the antibody does not react with mature oligodendrocytes.

15. The pharmaceutical composition of claim 10, wherein the antibody cross-reacts with spinal cord homogenate (SCH).

16. The pharmaceutical composition of claim 10, wherein the antibody primarily reacts with cells exhibiting a macrophage or microglial phenotype.

17. The pharmaceutical composition of claim 10, wherein the antibody is a monoclonal antibody.

18. The pharmaceutical composition of claim 10, wherein the antibody is a polyclonal antibody.

19. A method of stimulating remyelination of central nervous system axons comprising contacting the axons with an antibody directed against an epitope on glatiramer acetate (Copolymer 1) in an amount effective to stimulate remyelination of central nervous system axons.

20. The method of claim 19, wherein the antibody is a humanized antibody.

21. The method of claim 19, wherein the antibody is not cross-reactive with myelin basic protein (MBP).

22. The method of claim 19, wherein the antibody consists essentially of IgG1.

23. The method of claim 19, wherein the antibody does not react with mature oligodendrocytes.

24. The method of claim 19, wherein the antibody cross-reacts with spinal cord homogenate (SCH).

25. The method of claim 19, wherein the antibody primarily reacts with cells exhibiting a macrophage or microglial phenotype.

26. The method of claim 19, wherein the antibody is a monoclonal antibody.

27. The method of claim 19, wherein the antibody is a polyclonal antibody.

28. A method of treating a subject suffering from a disease associated with demyelination of central nervous system axons comprising administering to the subject an effective amount of an antibody directed against an epitope on glatiramer acetate (Copolymer 1) in an amount effective to treat the disease associated with demyelination of central nervous system axons.

29. The method of claim 28, wherein the antibody is a humanized antibody.

30. The method of claim 28, wherein the antibody is not cross-reactive with myelin basic protein (MBP).

31. The method of claim 28, wherein the antibody consists essentially of IgG1.

32. The method of claim 28, wherein the antibody does not react with mature oligodendrocytes.

33. The method of claim 28, wherein the antibody cross-reacts with spinal cord homogenate (SCH).

34. The method of claim 28, wherein the antibody primarily reacts with cells exhibiting a macrophage or microglial phenotype.

35. The method of claim 28, wherein the antibody primarily reacts with cells exhibiting a macrophage or microglial phenotype.

36. The method of claim 28, wherein the antibody is a monoclonal antibody.

37. The method of claim 28, wherein the antibody is a polyclonal antibody.

38. The method of claim 28, wherein the disease associated with demyelination of central nervous system axons is selected from the group consisting of: multiple sclerosis, acute disseminated encephalomyelitis, transverse myelitis, demyelinating genetic diseases, spinal cord injury, virus-induced demyelination, Progressive Multifocal Leucoencephalopathy, Human Lymphotrophic T-cell Virus I (HTLVI)-associated myelopathy, and nutritional metabolic disorders.

39. The method of claim 38, wherein the nutritional metabolic disorder is vitamin B_{12} deficiency.

40. The method of claim 38, wherein the nutritional metabolic disorder is central pontine myelinolysis.

41. The method of claim 28, wherein the effective amount is an amount from 0.5 mg/kg to 400 mg/kg.

42. The method of claim 41, wherein the effective amount is an amount from 0.5 mg/kg to 250 mg/kg.

43. A method of stimulating remyelination of central nervous system axons comprising contacting the axons with glatiramer acetate (Copolymer 1) in an amount effective

to stimulate remyelination of central nervous system axons.

44. A method of treating a subject suffering from a disease associated with demyelination of central nervous system axons comprising administering to the subject glatiramer acetate (Copolymer 1) in an amount effective to treat the disease associated with demyelination of central nervous system axons, wherein the disease associated with demyelination of central nervous system axons is selected from the group consisting of: acute disseminated encephalomyelitis, transverse myelitis, demyelinating genetic diseases, spinal cord injury, virus-induced demyelination, Progressive Multifocal Leucoencephalopathy, Human Lymphotrophic T-cell Virus I (HTLVI)-associated myelopathy, and nutritional metabolic disorders.

45. A method of stimulating proliferation of lymphocytes comprising contacting the lymphocytes with an antibody directed against an epitope on glatiramer acetate (Copolymer 1) in an amount effective to stimulate lymphocyte proliferation.

46. The method of claim 45, wherein the antibody is a humanized antibody.

47. The method of claim 45, wherein the antibody is not cross-reactive with myelin basic protein (MBP).

48. The method of claim 45, wherein the antibody consists

essentially of IgG1.

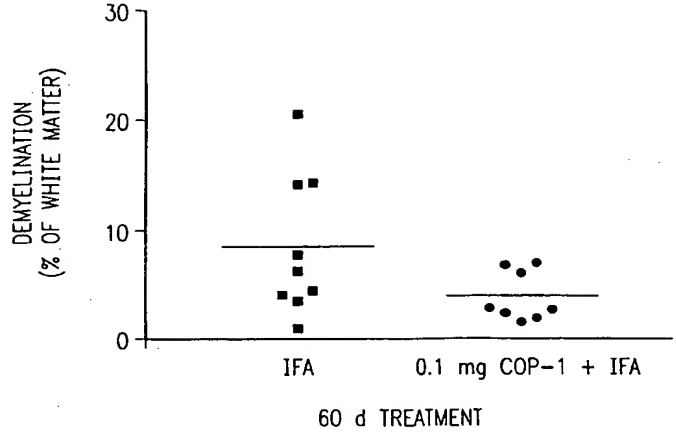
49. The method of claim 45, wherein the antibody does not react with mature oligodendrocytes.

50. The method of claim 45, wherein the antibody cross-reacts with spinal cord homogenate (SCH).

51. The method of claim 45, wherein the antibody primarily reacts with cells exhibiting a macrophage or microglial phenotype.

52. The method of claim 45, wherein the antibody is a monoclonal antibody.

53. The method of claim 45, wherein the antibody is a polyclonal antibody.


30
20
10
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(% OF WHITE MATTER)
IFA
0.1 mg COP-1 + IFA
60 d TREATMENT

FIG. 1

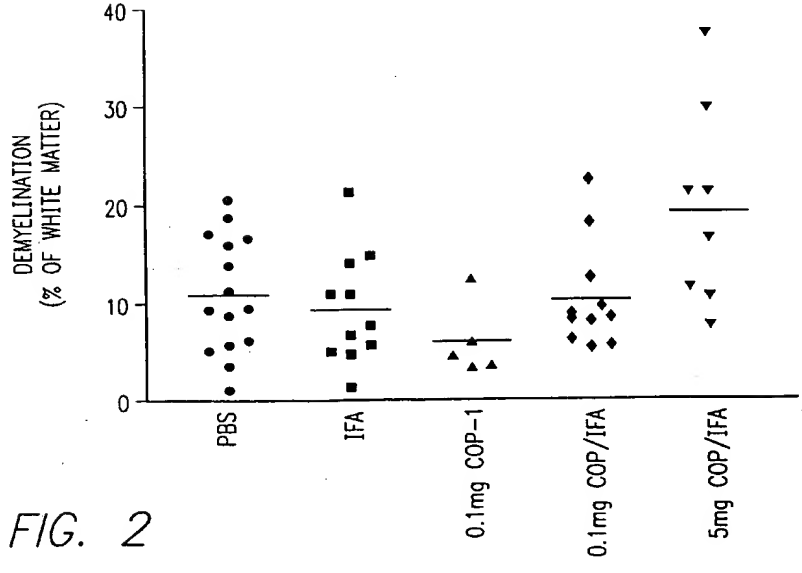
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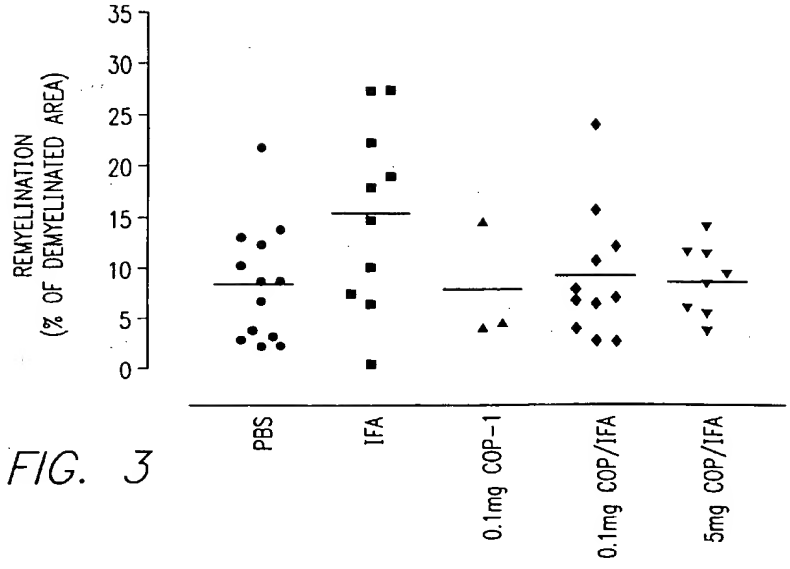
FIG. 2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(% OF DEMYELINATED AREA)
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30
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20
15
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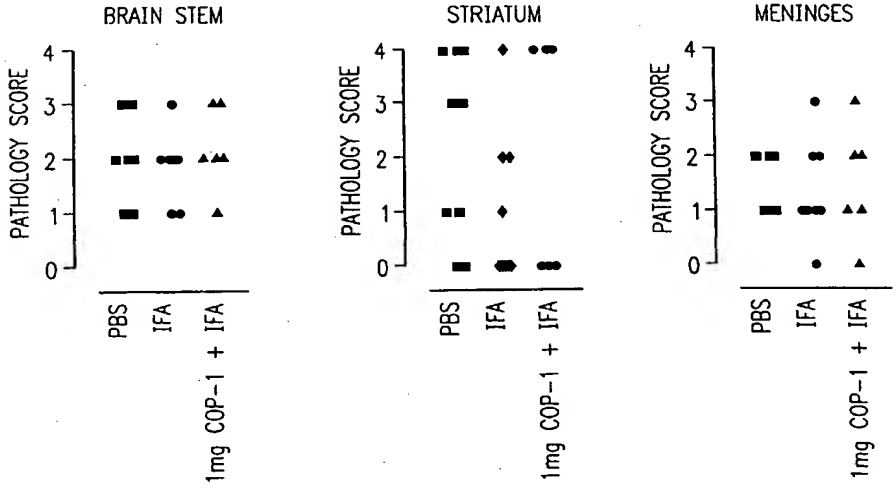
0.1mg COP/IFA
5mg COP/IFA

FIG. 3

FIG. 4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MENINGES
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IFA
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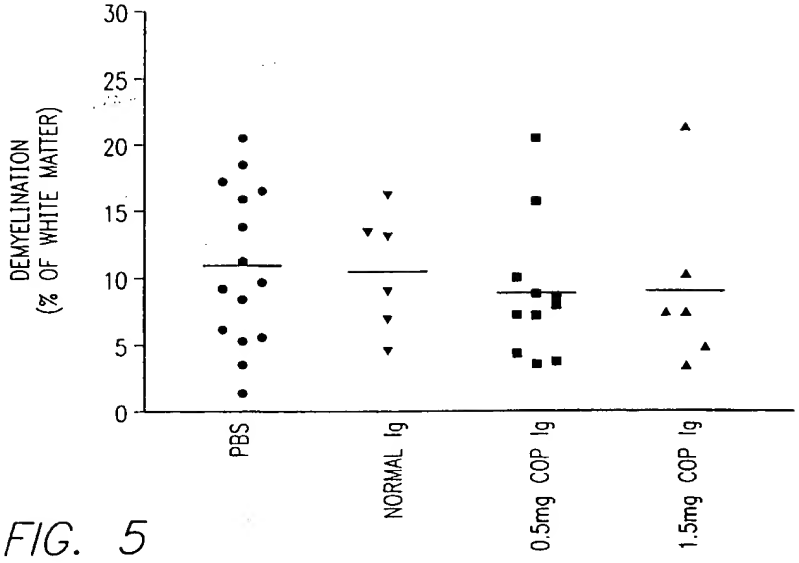
DEMYELINATION
(% OF WHITE MATTER)
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NORMAL Ig
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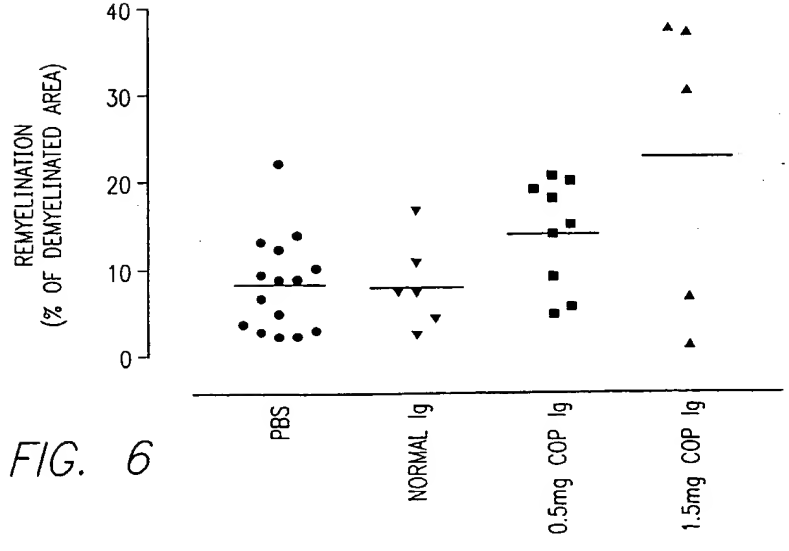
1.5mg COP Ig

FIG. 5


REMYELINATION
(% OF DEMYELINATED AREA)
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10
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PBS
NORMAL Ig
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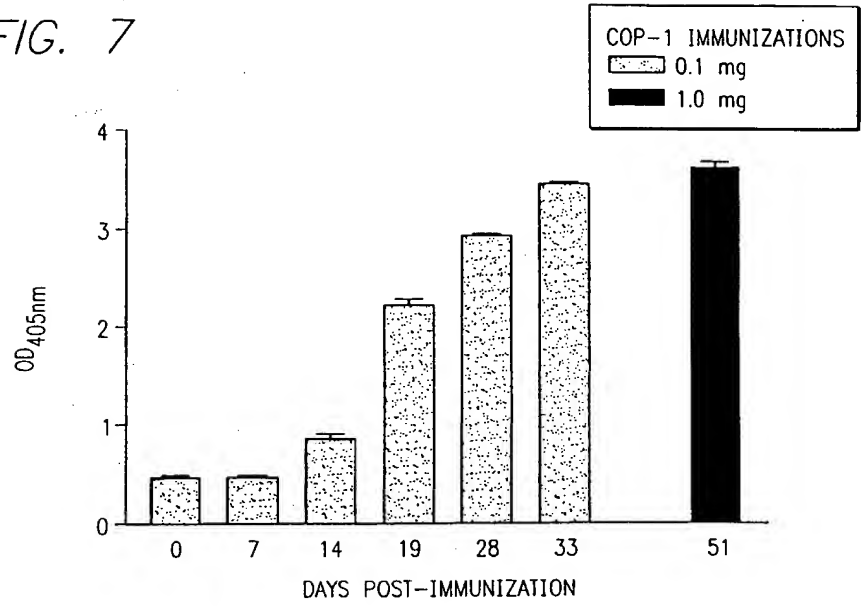
1.5mg COP Ig

FIG. 6

FIG. 7


COP-1 IMMUNIZATIONS
0.1 mg
1.0 mg
OD_{405nm}
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1
2
3
4
0
7
14
19
28
33
51
DAYS POST-IMMUNIZATION

FIG. 8

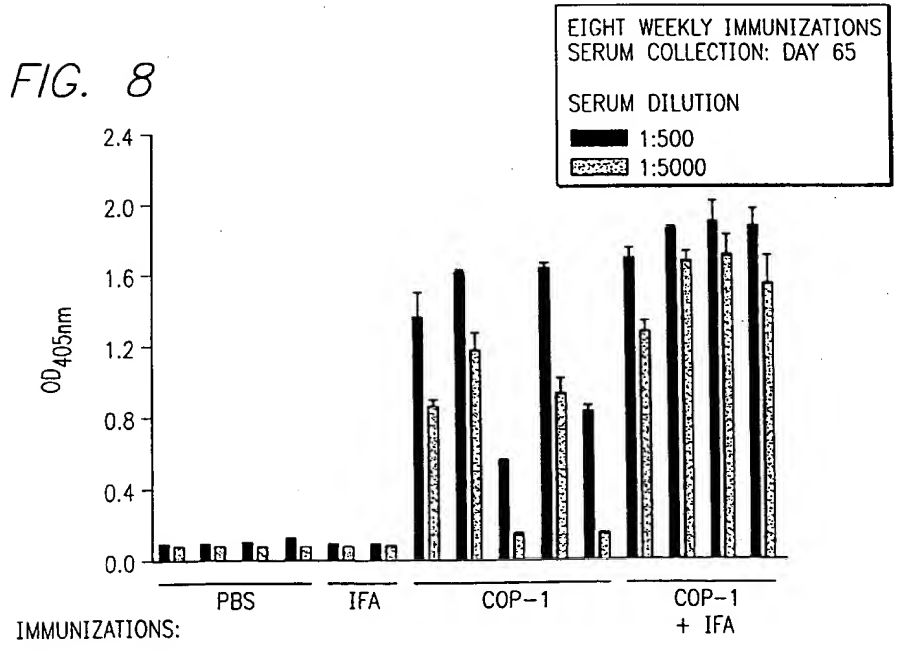
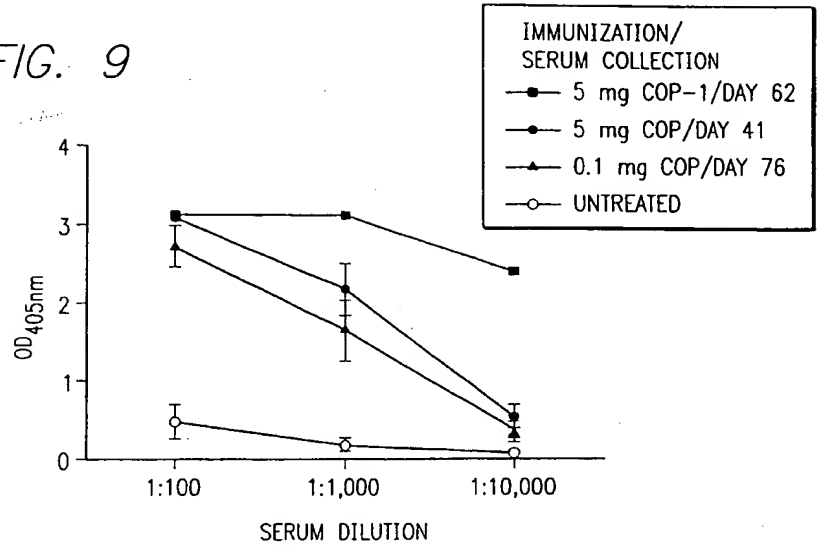

EIGHT WEEKLY IMMUNIZATIONS
SERUM COLLECTION: DAY 65
SERUM DILUTION
1:500
1:5000
OD_{405nm}
0.0
0.4
0.8
1.2
1.6
2.0
2.4
PBS
IFA
COP-1
COP-1
+ IFA
IMMUNIZATIONS:

FIG. 9


IMMUNIZATION/
SERUM COLLECTION
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0.1 mg COP/DAY 76
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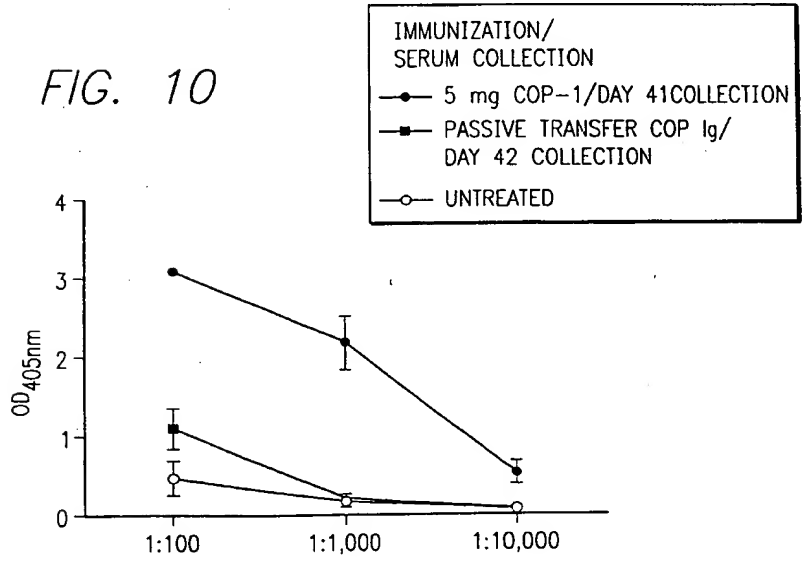
1
2
3
4
1:100
1:1,000
1:10,000
SERUM DILUTION

FIG. 10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PASSIVE TRANSFER COP Ig/
DAY 42 COLLECTION
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1
2
3
4
1:100
1:1,000
1:10,000
SERUM DILUTION

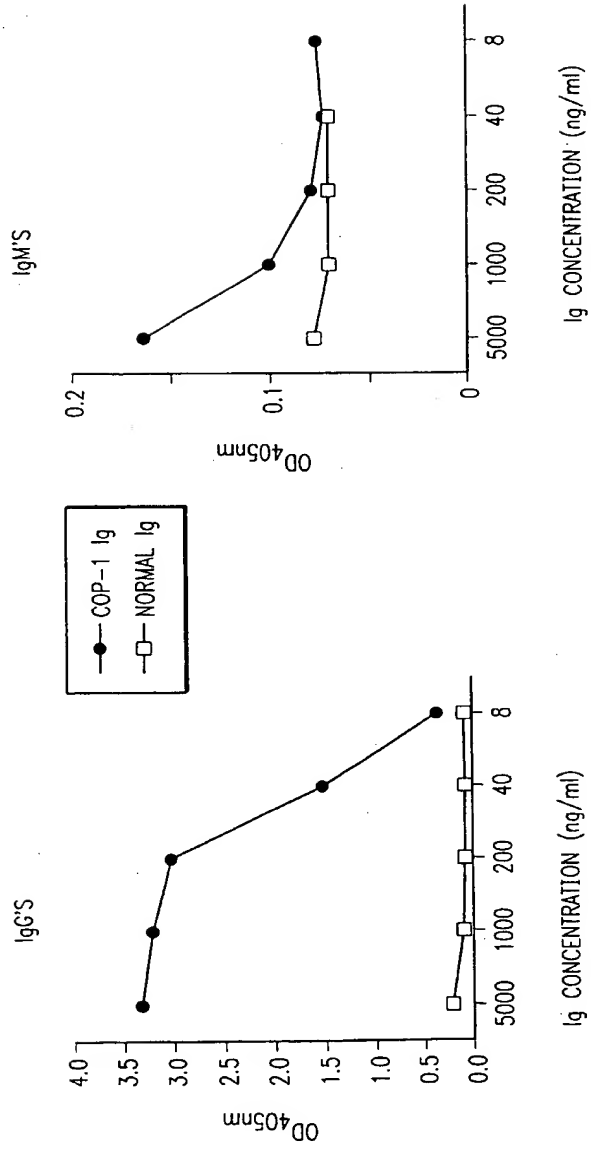
IgM'S
IgG'S
COP-1 Ig
NORMAL Ig
OD405nm
Ig CONCENTRATION (ng/ml)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
0
0.1
0.2
8
40
200
1000
5000

FIG. 11

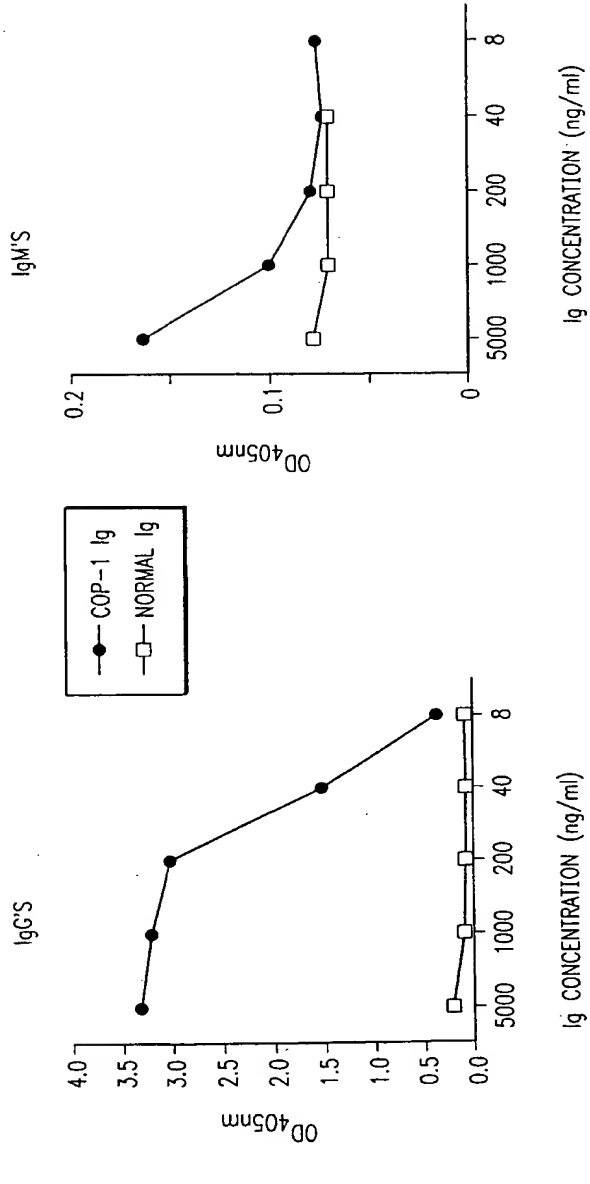
IgG'S
IgM'S
COP-1 Ig
NORMAL Ig
OD405nm
Ig CONCENTRATION (ng/ml)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
0
0.1
0.2
5000
1000
200
40
8

FIG. 11

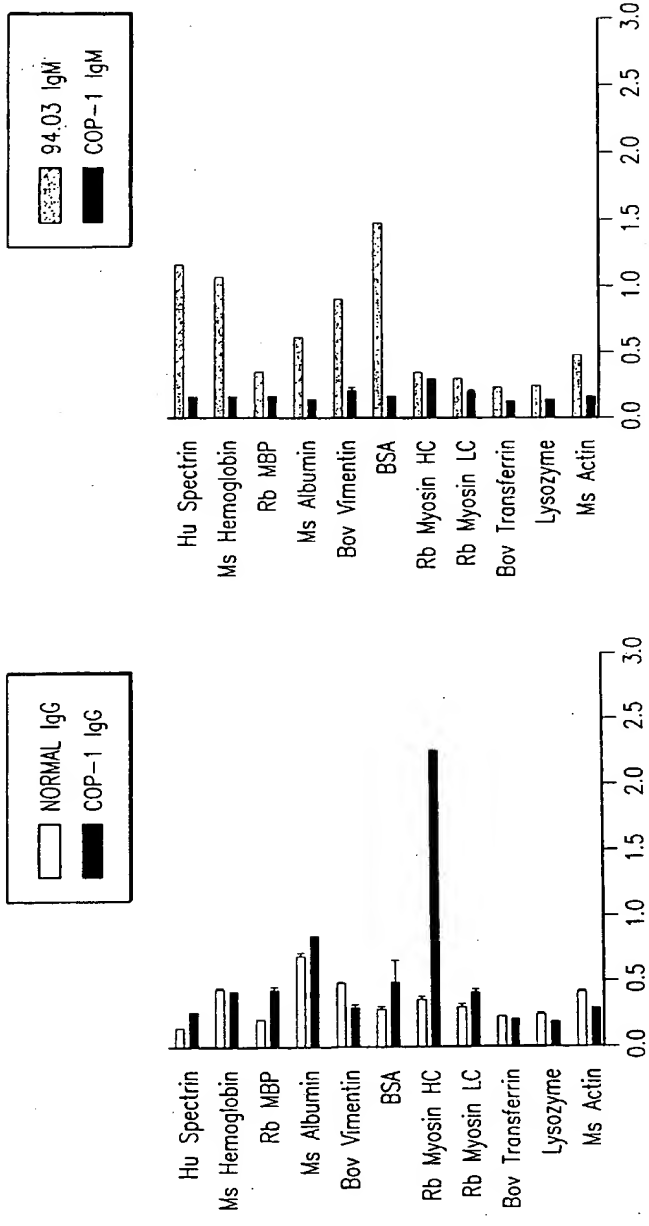
NORMAL IgG
COP-1 IgG
94.03 IgM
COP-1 IgM
Hu Spectrin
Ms Hemoglobin
Rb MBP
Ms Albumin
Bov Vimentin
BSA
Rb Myosin HC
Rb Myosin LC
Bov Transferrin
Lysozyme
Ms Actin
0.0
0.5
1.0
1.5
2.0
2.5
3.0
OD405nm

FIG. 12

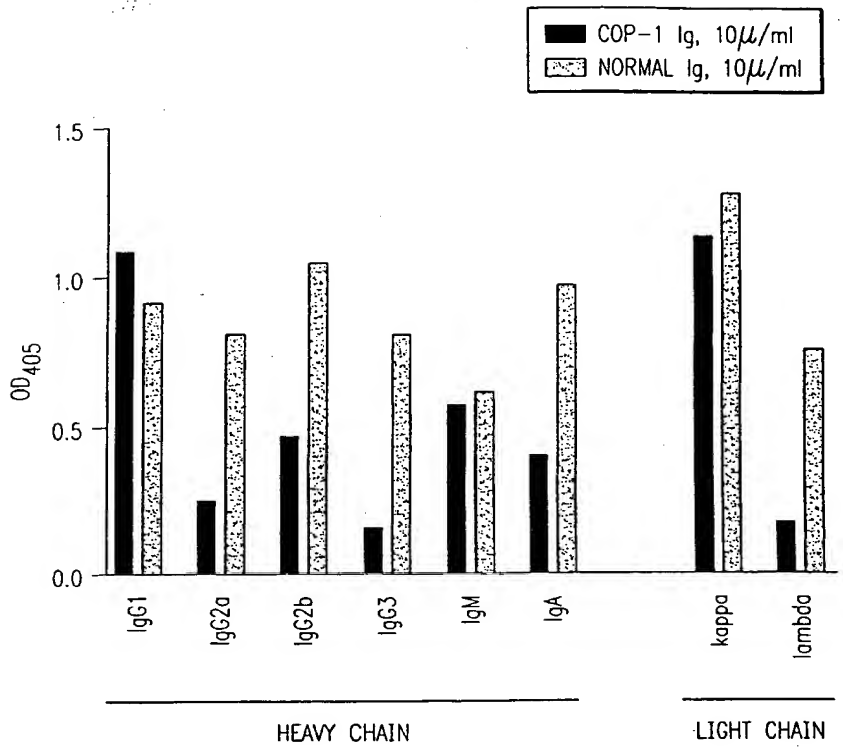
COP-1 Ig, 10μ/ml
NORMAL Ig, 10μ/ml
1.5
1.0
0.5
0.0
OD405
IgG1
IgG2a
IgG2b
IgG3
IgM
IgA
kappa
lambda
HEAVY CHAIN
LIGHT CHAIN

FIG. 13

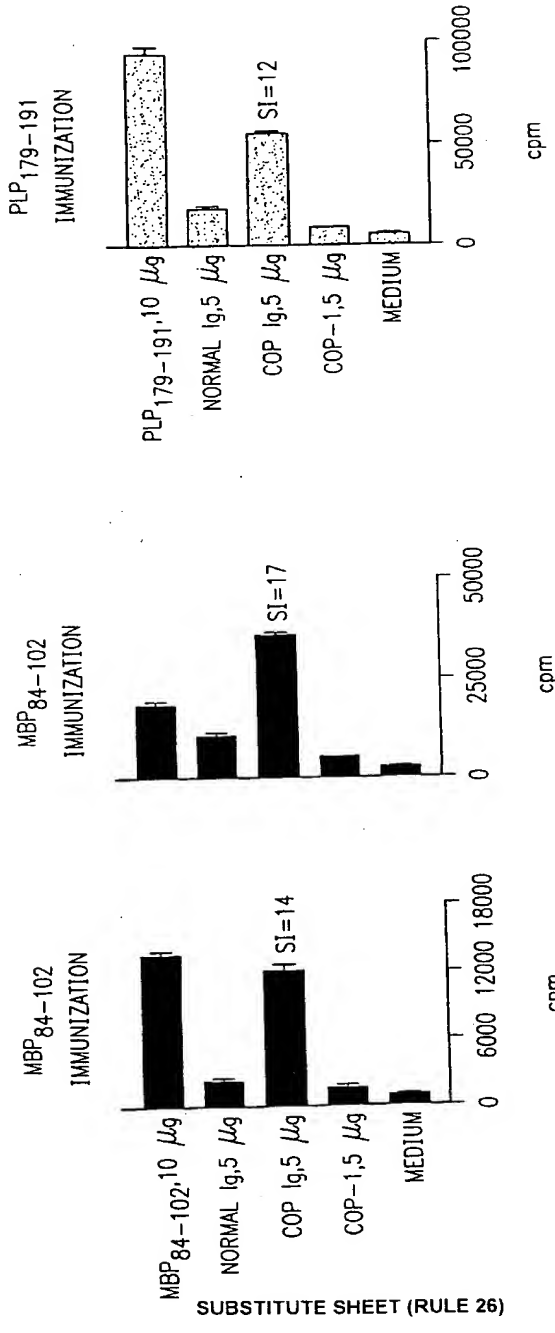
MBP84-102 IMMUNIZATION
MBP84-102,10 μg
NORMAL Ig,5 μg
COP Ig,5 μg
COP-1,5 μg
MEDIUM
SI=14
0 6000 12000 18000
cpm
MBP84-102 IMMUNIZATION
SI=17
0 25000 50000
cpm
PLP179-191 IMMUNIZATION
PLP179-191,10 μg
NORMAL Ig,5 μg
COP Ig,5 μg
COP-1,5 μg
MEDIUM
SI=12
0 50000 100000
cpm

FIG. 14

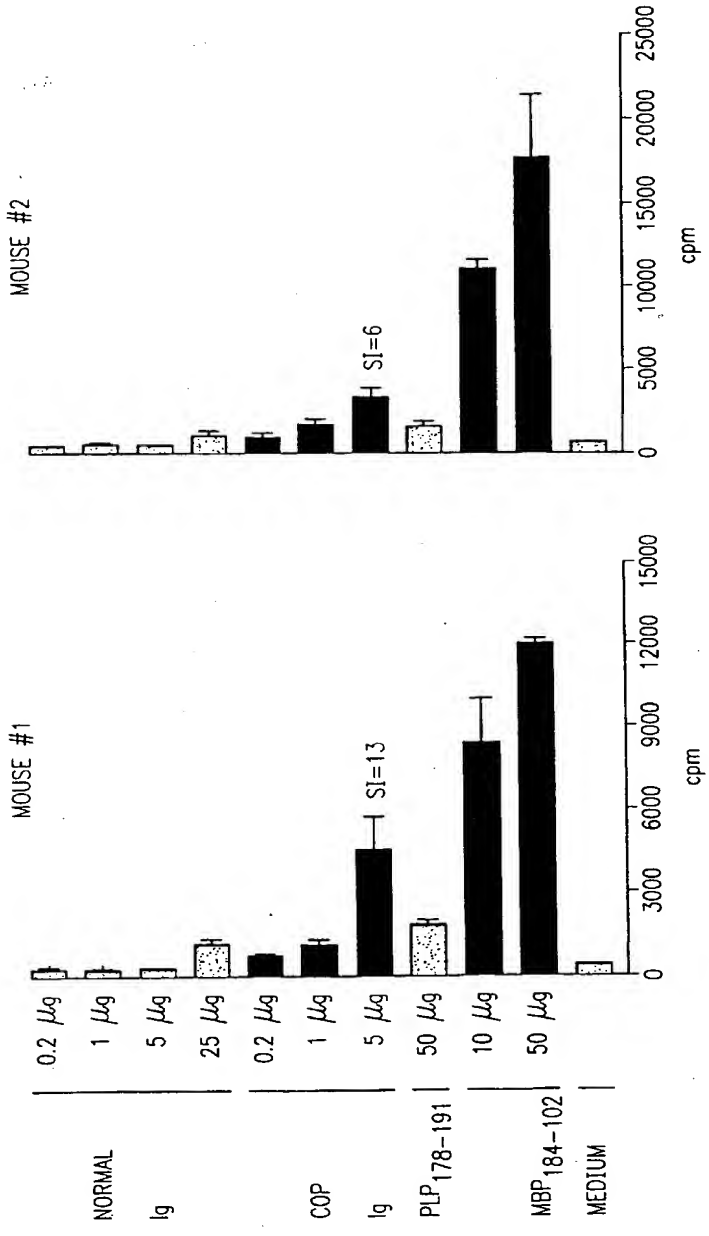
MOUSE #2
MOUSE #1
SI=6
SI=13
cpm
0
5000
10000
15000
20000
25000
3000
6000
9000
12000
15000
0.2 μg
1 μg
5 μg
25 μg
0.2 μg
1 μg
5 μg
50 μg
10 μg
50 μg
NORMAL
Ig
COP
Ig
PLP178-191
MBP184-102
MEDIUM

FIG. 15

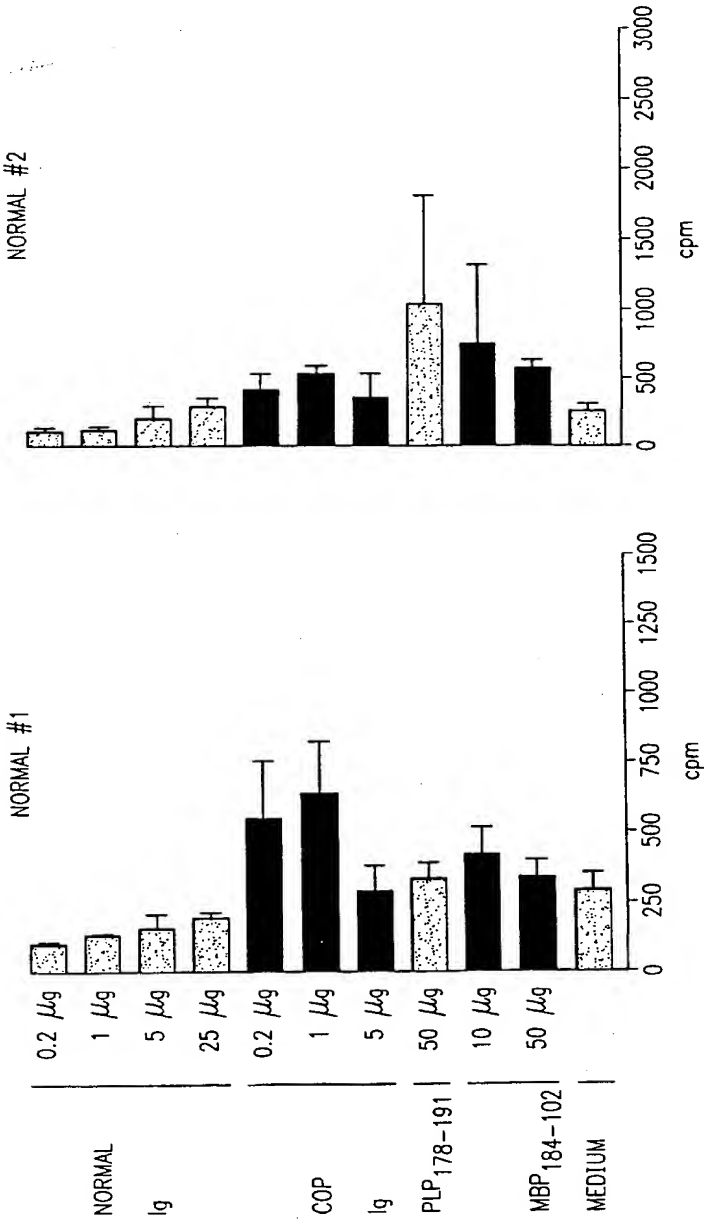
NORMAL #2
NORMAL #1
cpm
0
500
1000
1500
2000
2500
3000
250
750
1250
0.2 μg
1 μg
5 μg
25 μg
0.2 μg
1 μg
5 μg
50 μg
10 μg
50 μg
NORMAL
Ig
COP
Ig
PLP178-191
MBP184-102
MEDIUM

FIG. 16

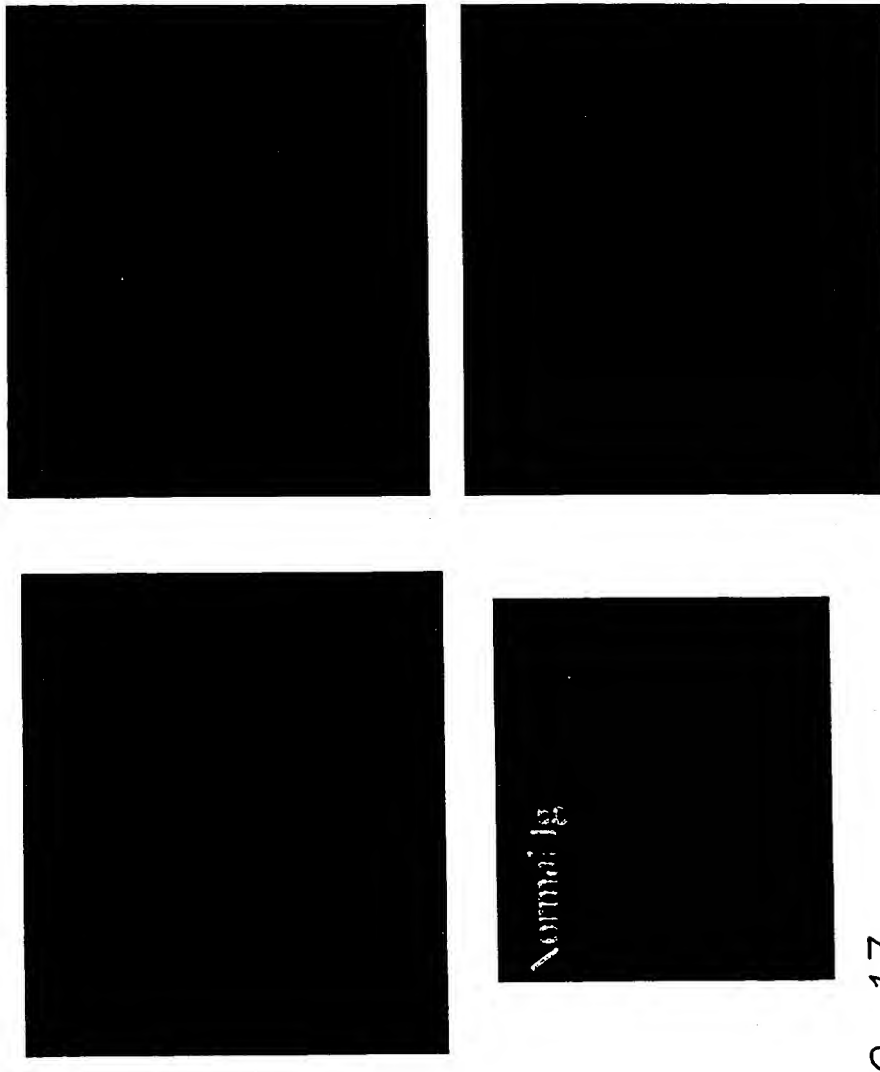

FIG. 17

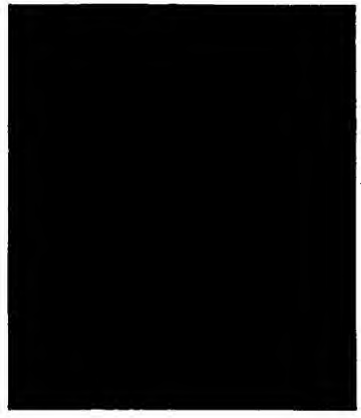

FIG. 18E

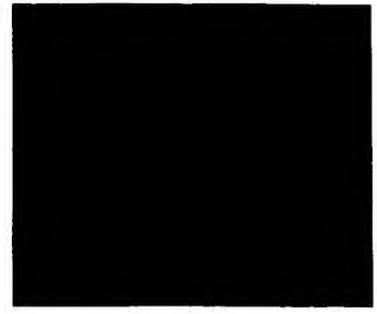

FIG. 18F

FIG. 18C

FIG. 18D

FIG. 18A

FIG. 18B

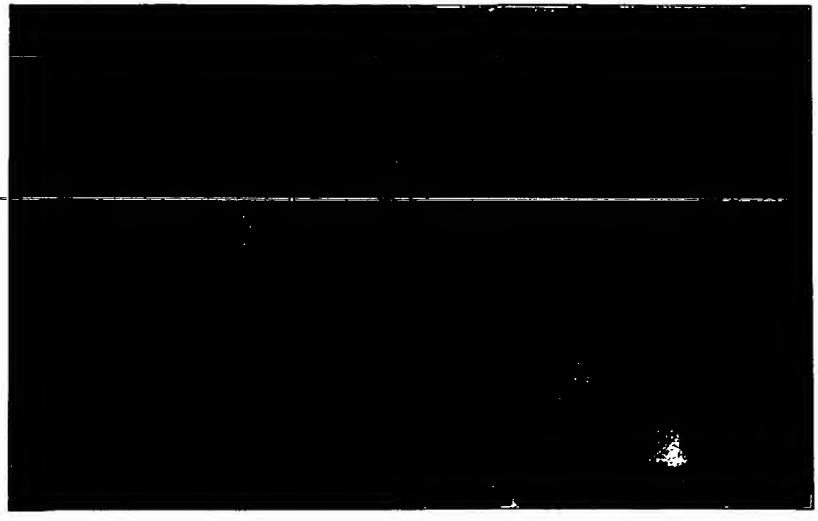

FIG. 19

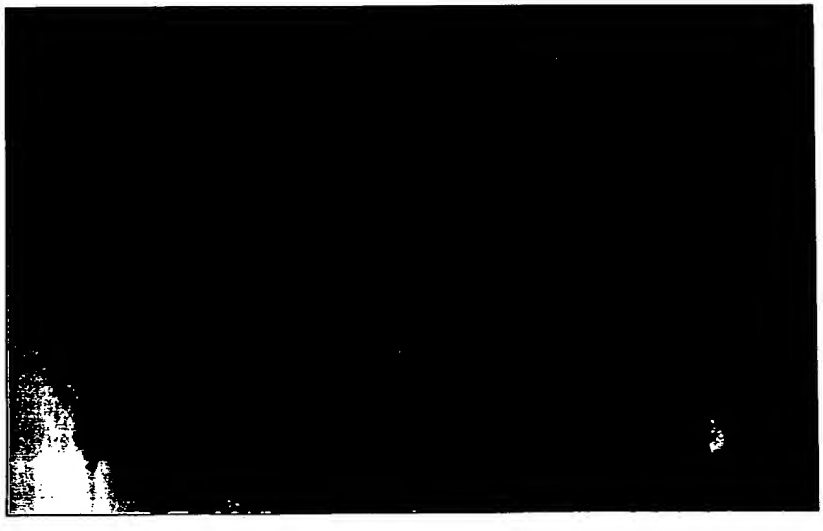

FIG. 20

FIG. 21

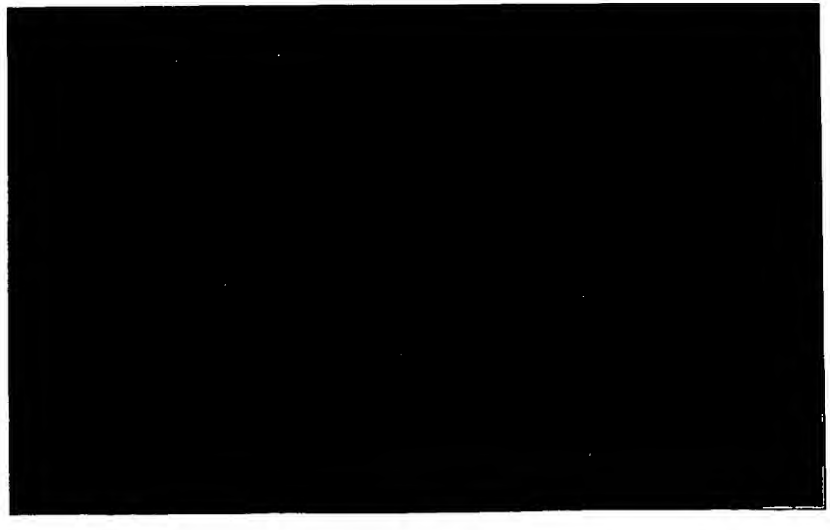

FIG. 22

FIG. 23

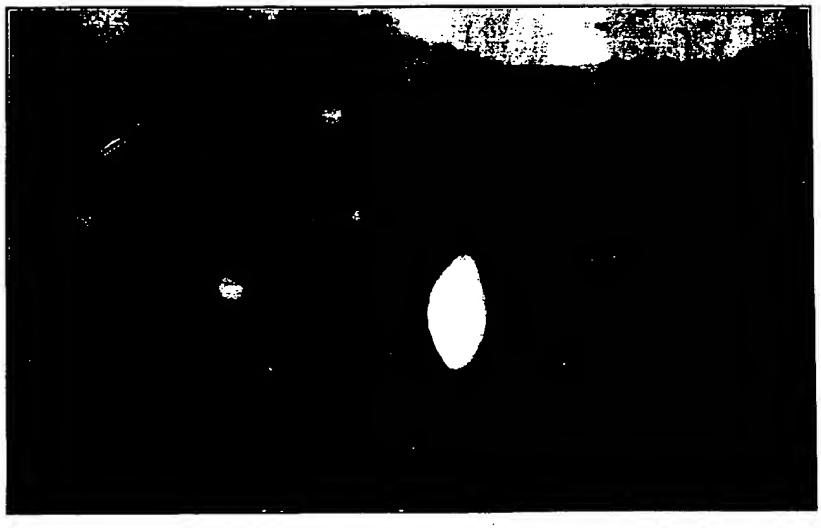

FIG. 24

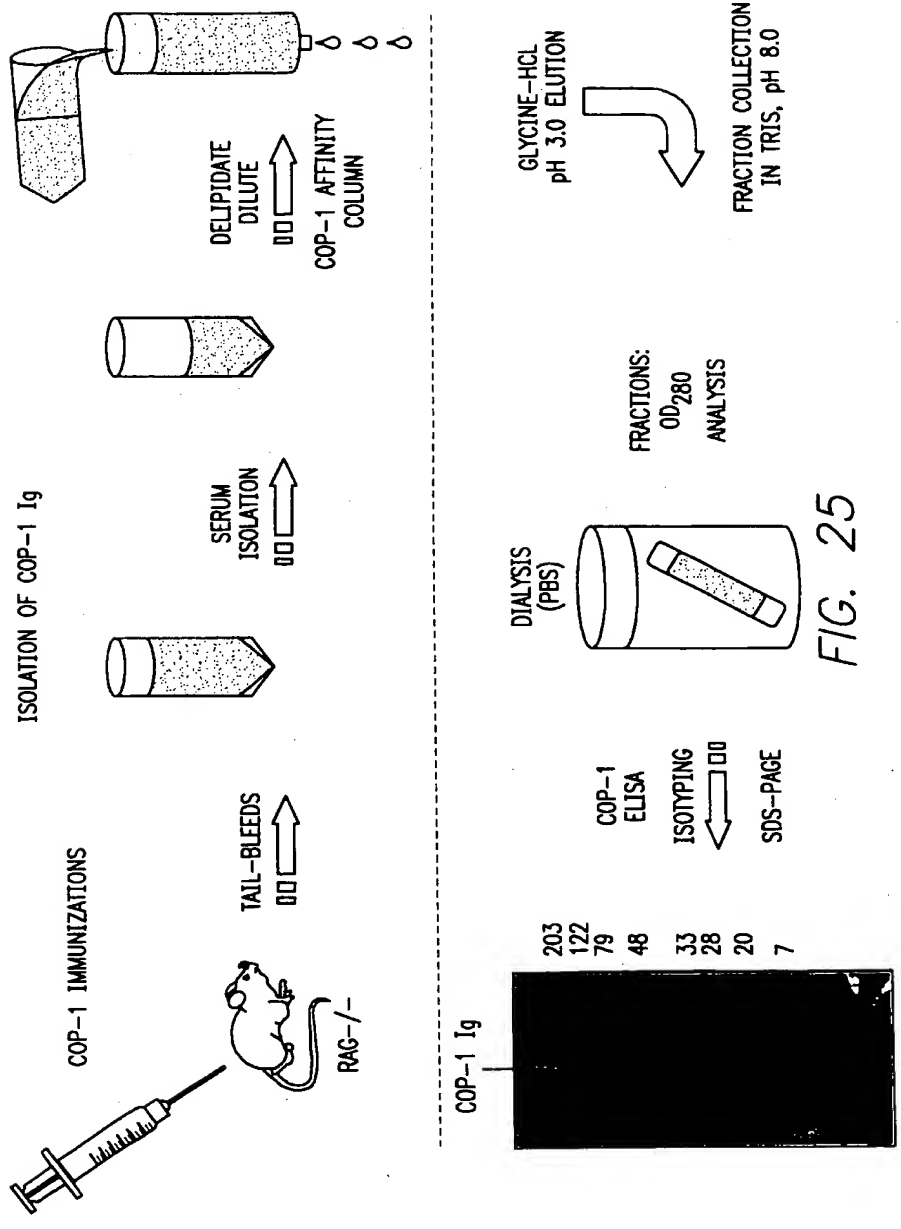
ISOLATION OF COP-1 Ig
COP-1 IMMUNIZATIONS
RAG-/-
TAIL-BLEEDS
SERUM ISOLATION
DELIPIDATE DILUTE
COP-1 AFFINITY COLUMN
GLYCINE-HCL pH 3.0 ELUTION
FRACTION COLLECTION IN TRIS, pH 8.0
DIALYSIS (PBS)
FRACTIONS: OD_{280} ANALYSIS
COP-1 ELISA
ISOTYPING
SDS-PAGE
COP-1 Ig
203
122
79
48
33
28
20
7

FIG. 25

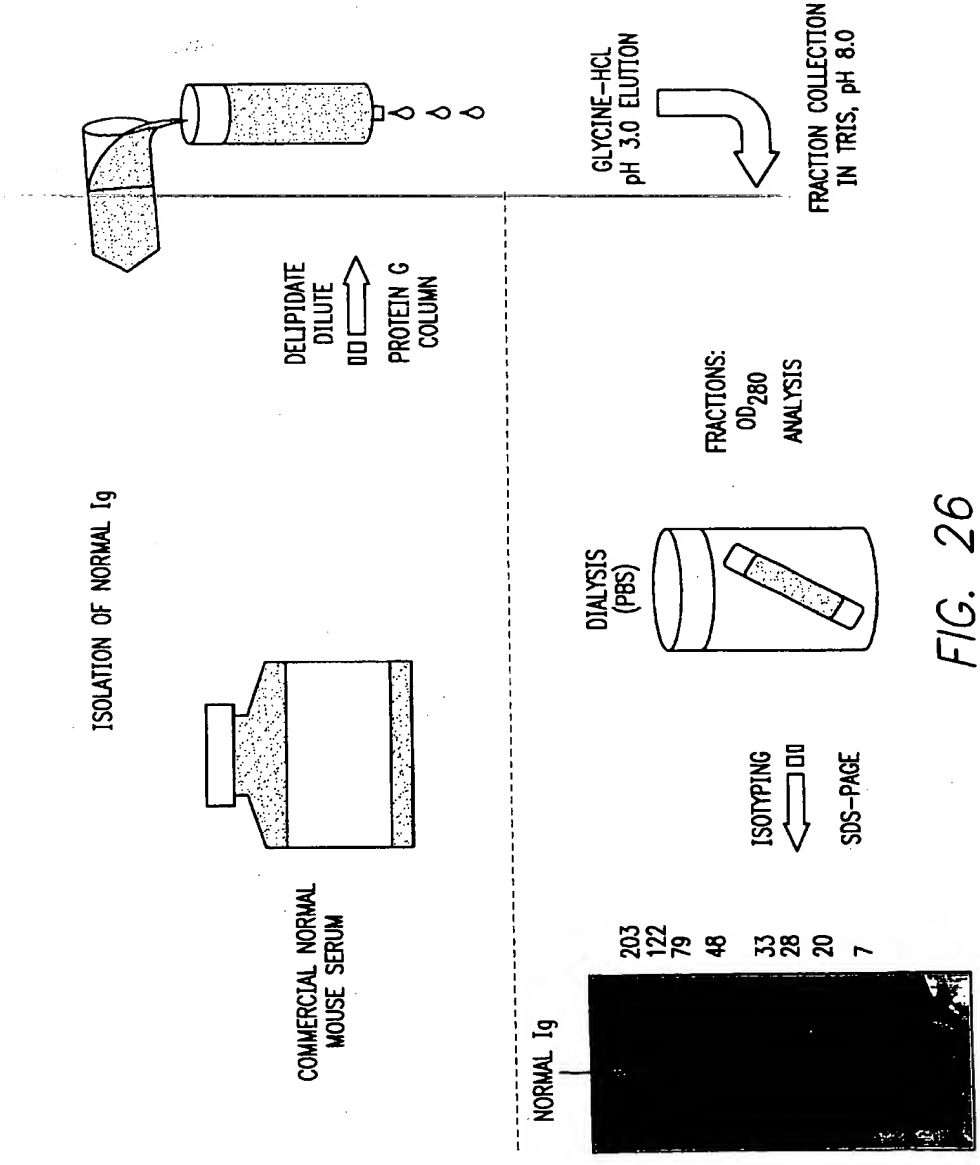
ISOLATION OF NORMAL Ig
COMMERCIAL NORMAL MOUSE SERUM
DELIPIDATE DILUTE
PROTEIN G COLUMN
GLYCINE-HCL pH 3.0 ELUTION
FRACTION COLLECTION IN TRIS, pH 8.0
DIALYSIS (PBS)
FRACTIONS: OD_{280} ANALYSIS
ISOTYPING
SDS-PAGE
NORMAL Ig
203
122
79
48
33
28
20
7

FIG. 26

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/19584

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A61K 39/40, 39/42, 39/395; C07K 16/00; C12N 5/00, 5/02; C12P 21/08

US CL : 424/133.1, 139.1, 141.1; 435/375; 530/387.1, 398.3, 388.1, 389.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/133.1, 139.1, 141.1; 435/375; 530/387.1, 398.3, 388.1, 389.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, BIOSIS, CAPLUS, USPATFULL, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PURI, J. et al. Modulation of the Immune Response in Multiple Sclerosis. J. Immunology. 1997, Vol. 158, pages 2471-2476.	1-53
A	FRIDKIS-HARELI, M. et al Binding of copolymer 1 and myelin basic protein leads to clustering of class II MHC molecules on antigen-presenting cells. International Immunology. 1997, Vol. 9, No. 7, pages 925-934.	1-53
A	TEITELBAUM, D. et al. Cross-reactions and specificities of monoclonal antibodies against myelin basic protein and against the synthetic copolymer 1. Proc. Natl. Acad. Sci. USA. November 1991,Vol. 88, pages 9528-9532.	1-53

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 01 OCTOBER 2001	Date of mailing of the international search report 15 NOV 2001
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer OLGA N. CHERNYSHEV Telephone No. (703)-308-0196

Form PCT/ISA/210 (second sheet) (July 1998)*